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APPEAL BRIEF

Applicant	:	Shinichiro Morita et al.
App. No	:	10/070,938
Filed	:	June 4, 2002
For	:	MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND METHOD FOR REGENERATING CARDIOVASCULAR TISSUE
Examiner	:	David M. Naff
Art Unit	:	1651

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In accordance with the Notice of Appeal filed June 8, 2007, Applicant submits this Appeal Brief.

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I. REAL PARTIES IN INTEREST

Pursuant to 37 C.F.R. § 1.192, Appellants hereby notify the Board of Patent Appeals and Interferences that the real parties in interest are the assignees of this application: Gunze, Ltd., 1, Zeze, Aono-cho, Ayabe-shi, Kyoto 623-0051, Japan: and Tokyo Women's Medical University, 8-1, Kawada-cho, Shinjuku-ku, Tokyo 162-0054, Japan.

II. RELATED APPEALS AND INTERFERENCES

Appellants are unaware of any related appeals or interferences.

III. STATUS OF CLAIMS

The above-identified application was filed with 11 claims. Claims 1-11 were rejected by the Examiner in an Office Action mailed September 8, 2004. Subsequently, Claims 12-14 were added. Claims 1-14 were finally rejected in an Office Action mailed May 17, 2005. Subsequent to that Office Action, Claims 1-6 and 12-14 were cancelled and Claims 15-19 were added. The new claims were entered for purposes of appeal by the Examiner in an Advisory Action mailed October 12, 2005, in which the Examiner indicated that Claims 7-11 and 15-19 remained rejected. Applicant filed an Appeal Brief on March 17, 2006. The Examiner subsequently reopened prosecution, again rejecting Claims 7-11 and 15-19 in a non-final Office Action mailed June 1, 2006. Claim 7 was subsequently amended. Claims 7-11 and 15-19 were finally rejected in an Office Action mailed March 12, 2007. Accordingly, Claims 7-11 and 15-19 are the subject of this appeal. The claims are attached hereto as Section VIII.

IV. STATUS OF AMENDMENTS

All amendments have been entered.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed subject matter relates to Appellant's discovery of a matrix for culturing cardiovascular cells to regenerate cardiovascular tissue. The matrix includes a sponge made of a bioabsorbable material that is reinforced with a bioabsorbable material, which reinforcement is integrated with the sponge and located inside or on the exterior surface of the matrix. The sponge allows cells seeded thereon to adhere firmly thereto, enables the matrix to be absorbed *in vivo* once the blood vessel is regenerated, and allows the matrix to maintain sufficient strength to

permit blood flow until the blood vessel is regenerated *in vivo*. See, e.g., specification at p. 3, lines 9-20.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The Examiner has rejected Claims 7-11 and 15-19 under 35 U.S.C. § 112, second paragraph as being indefinite.

The Examiner has rejected Claims 7-9, 11 and 15-19 under 35 U.S.C. § 103(a) as being unpatentable over Vacanti, et al., United States Patent No. 5,855,610, in view of Vyakarnam, et al., United States Patent No. 6,534,084 and Morita, Japanese Patent No. 3-23864.

The Examiner has rejected Claim 10 under 35 U.S.C. § 103(a) as being unpatentable over Vacanti '610, and further in view of Fofonoff et al., United States Patent No. 5,882,929, taken with Cox, United States Patent No. 6,719,789, or Love, United States Patent No. 5,509,930.

The Examiner has rejected Claims 7, 8, and 11 under 35 U.S.C. § 103(a) as being unpatentable over Naughton, et al., United States Patent No. 5,863,531.

The Examiner has rejected Claims 9, 15, and 19 under 35 U.S.C. § 103(a) as being unpatentable over Naughton '531 and further in view of Vacanti '610.

The Examiner has rejected Claim 10 under 35 U.S.C. § 103(a) as being unpatentable over Naughton '531 and Vacanti '610, and further in view of Fofonoff '929 taken with Cox '789 or Love '930.

The Examiner has rejected Claims 16-18 under 35 U.S.C. § 103(a) as being unpatentable over Naughton '531 and Vacanti '610, and further in view of Vyakarnam '084, and if necessary in further view of Morita '864.

VII. ARGUMENT

Engineered cardiovascular grafts are known in which cells are seeded onto a fibrous or sponge-like material and grown on the material before implantation. The Examiner has cited several references disclosing such grafts, and none indicated that the grafts would experience any problems after implantation. Applicants have discovered, quite unexpectedly in view of the cited prior art, that when an unreinforced material is employed, the resulting graft can fail

catastrophically. The solution Applicants came up with is admittedly quite simple: reinforce the material with a separate biodegradable reinforcement prior to seeding cells on it.

The Examiner erroneously focuses on the simplicity of the solution rather than the nonobviousness of the problem. This has led him to overstate the teachings of the cited references, impermissibly employ hindsight reconstruction using Applicant's disclosure as a template, and completely ignore Applicants' discovery of the failure problem, which is a significant contribution to the tissue engineering art.

A. Claims 7-9, 11 and 15-19 under 35 U.S.C. § 103(a) Are Not Obvious Over the Combination of Vacanti '610, Vyakarnam '084, and Morita '864

The Examiner has rejected Claims 7-9, 11, and 15-19 under 35 U.S.C. § 103(a) as obvious over Vacanti '610, in view of Vyakarnam '084 and Morita '864. The Examiner bases this rejection on allegations that

(A) the problem of failure of unreinforced grafts described in Applicants' specification did not exist in the prior art, because the unreinforced prior art materials were stronger than those described in Applicants' specification;

(B) one of skill in the art would have a motivation to combine the references, despite the fact that Morita does not relate to tissue culture, and neither Naughton nor Vyakarnam disclose or suggest that a matrix to be used for culturing cardiovascular tissue should be reinforced using a bioabsorbable material; and

(C) Applicant's disclosure of the unexpected effect that a cardiovascular graft produced according to the teachings of the prior art failed rapidly whereas one produced in accordance with the claimed invention was successful is unpersuasive, because "it would have been obvious to provide reinforcement."

Each of these allegations is addressed below.

1. Applicants' Discovery of the Catastrophic-Failure Problem Is Strong Evidence of Nonobviousness Discounted by the Examiner

Since Eibel Process Co. v. Minnesota & Ontario Paper Co., 261 U.S. 45, 68 (1923), courts have recognized that an inventor's discovery of the source of a problem and how to remedy it is strong evidence of the nonobviousness of the resulting invention. This reasoning

applies all the more when it is not merely the source of or solution to a problem, but rather the problem itself, that is unknown. In re Nomiya, 509 F.2d 566, 572 (C.C.P.A. 1975) ("If . . . there is no evidence of record that a person of ordinary skill in the art at the time of appellants' invention would have expected the problem . . . to exist at all, it is not proper to conclude that the invention which solves this problem . . . would have been obvious to that hypothetical person of ordinary skill in the art."); see also In re Cable, 347 F.2d 872, 878 (C.C.P.A. 1965) (holding claims nonobvious where none of the cited references "suggest the problem or solution contemplated by applicant"); In re Shaffer, 229 F.2d 476, 480 (C.C.P.A. 1956) (holding references "improperly combined" where "a person having the references before him who was not cognizant of appellant's disclosure would not be informed that the problem solved by appellant ever existed"). Indeed, "a patentable invention may lie in the discovery of the source of a problem even though the remedy may be obvious once the source of the problem is identified." In re Sponnoble, 405 F.2d 578, 585 (C.C.P.A. 1969). In this case, Applicants clearly discovered the existence of a problem not recognized by the cited prior art.

The main cited references, Vacanti '610 and Vyakarnam '084, disclose an unreinforced biodegradable fiber-based material for use in the production of cardiovascular graft tissue. See Vacanti '610 at col. 7, line 58 – col. 9, line 16 (polyglycolic acid fiber based matrix); Vyakarnam '084 at col. 20, line 7 – col. 21, line 47 (polymerized ε-caprolactone/glycolide-based foam). Neither reference suggests that reinforcement of these materials is required for the resulting grafts to have sufficient strength for use. In fact, Vacanti suggests that heart valves engineered using the unreinforced matrix had sufficient strength for use in patients. See Vacanti '610 at col. 8, lines 19-23.

Applicants were apparently the first to discover that this was wrong and that reinforcement of the starting material was required to avoid failure of the graft. As described in Applicants' specification, a cellular sponge-like material comprising L-lactide-caprolactone copolymer was used as a starting material for an engineered cardiovascular graft. Specification at p. 12. Grafts were produced using both the unreinforced material and material that had been reinforced using a poly-L-lactide fiber-based material formed in a double cylinder. Id. at pp. 12-

13. Surprisingly, in view of the prior art, the graft produced using the unreinforced material failed in one week after implantation into a dog's vena cava. Id. at p. 13. In contrast, the graft produced using the reinforced material remained intact and exhibited significant tissue ingrowth six months after implantation. Id. In short, Applicants discovered a fact apparently unknown in the tissue engineering field: cardiovascular tissue grown on unreinforced fiber-based materials was subject to catastrophic failure even if the tissue growth was apparently complete before implantation.

The Examiner responds to this evidence by suggesting that the problem Applicants discovered does not, in fact, exist. He states that Vacanti's "fiber-based matrix" has "sufficient strength" without reinforcement, because "fibers in one part of the mesh apparently acted as a reinforcement for fibers in another part of the mesh." Office Action at 9. The Examiner distinguishes Applicants' unreinforced material from the "fiber-based matrix" employed by Vacanti. "[W]hen the plain-weave cloth is omitted as reinforcement, there are no fibers present as contained by the fiber mesh of Vacanti et al. Obviously, the non-reinforced matrix used for comparison in Example 1 will not have the strength of the fiber mesh used in the examples of Vacanti et al." Office Action at 9. The Examiner cites absolutely no support for this proposition. Apparently it is based on his own technical knowledge. There is certainly no basis for it in the cited references.

Indeed, the cited references themselves contradict the Examiner's view. Vacanti actually equates devices that employ "fibers" with those that are "sponge-like," considering them all to be "fibrous" and employable in the disclosed matrix. See Vacanti '610 at col.3, lines 49-51 ("As used herein, 'fibrous' includes one or more fibers that is entwined with itself, multiple fibers in a woven or non-woven mesh, and sponge like devices."). The foam disclosed in Vyakarnam has a similar sponge-like structure, see Vyakarnam '084 at Fig. 1, and would also be a "fibrous" material under Vacanti's definition. Vacanti is clearly concerned with providing sufficient mechanical strength in the grafts, see Vacanti '610 at col. 2, lines 37-42, and yet identifies no great differences in strength between the various unreinforced fibrous materials, including sponge-like materials such as those used in Applicants' comparative example. Vacanti implies

thereby that all of the fibrous materials he discloses have sufficient strength. The Examiner's contrary position, that the fibrous materials "obviously" have significantly different strengths, is thus not merely unsupported but contradicted by the very references he cites.

It is axiomatic that prior art references must be considered as a whole, including those disclosures that would support a conclusion of nonobviousness. See W.L. Gore & Assocs., Inc. v. Garlock, Inc., 721 F.2d 1540, 1551 (Fed. Cir. 1983). The Examiner has baselessly assumed that Vacanti's material is so much stronger than Applicants' that no further reinforcement would be needed. He has substituted that assumption for disclosures in the prior art documents that strongly suggest there is no such difference in strength in the materials. He is not entitled to do so.

Some courts have held claims obvious despite allegations of discovery of an unknown problem. See Solder Removal Co. vs. U.S. Int'l Trade Comm'n, 582 F.2d 628, 635 (C.C.P.A. 1978); In re Gershon, 372 F.2d 535, 538-39 (C.C.P.A. 1967). However, in those cases there has been no compelling evidence for the existence of the problem. In this case, Applicants' specification itself establishes that engineered grafts produced using the same unreinforced materials preferably employed by the cited prior art references failed, while the reinforced graft remained functional. The Examiner is not entitled to ignore this evidence, which establishes the nonobviousness of the claims. See Solder Removal Co., 582 F.2d at 635 (noting that it is "incorrect" to view "arguments that an invention solved a problem not previously recognized, and that nonobviousness may be evidenced by discovery of a problem source, as irrelevant").

2. The Examiner has Failed to Establish a *Prima Facie* Case of Obviousness, Because There is No Apparent Reason to Combine the Reference Teachings

The Supreme Court has recently clarified the law governing obviousness determinations. See KSR Int'l Co. v. Teleflex, Inc., 127 S.Ct. 1727 (2007). The Court stated that as part of the obviousness inquiry, it is necessary to "determine whether there was an apparent reason to combine the known elements in the fashion claimed." KSR Int'l Co., 127 S.Ct. at 1731; see also Ex parte Smith, No. 2007-1925 (Bd. Pat. App. & Interf. June 25, 2007) (precedential opinion adopting KSR standard in ex parte prosecution context). In this case, there was no such apparent

reason to combine Vacanti '610 and Vyakarnam '084, which relate to tissue engineering, with Morita '864, which relates to an artificial implant on which no tissue is engineered before implantation.

Claim 7, the only pending independent claim, contains the following limitation: "the reinforcement being integrated with the sponge and located inside or on the exterior surface of the matrix." The Examiner identifies such a reinforcement in the disclosures of Vacanti '610, Vyakarnam '084, and Morita '864. See Office Action at 6-10. When properly understood, however, neither Vacanti nor Vyakarnam disclose or suggest such a reinforcement, and there is no reason to combine the reinforcement of Morita, which has nothing to do with ex vivo tissue engineering, with either Vacanti or Vyakarnam.

a. Vacanti's "Struts" Are Neither Integral With The Sponge Nor Inside or on the Exterior Surface of the Matrix

As noted by the Examiner, see id at 6-7, the only disclosure in Vacanti that corresponds to the claimed reinforcements are the "struts," which appear to be discussed only at col. 3, lns. 62-67 and col. 5, lns. 34-48 of the reference. The struts are described as imparting "resistance to mechanical forces", thereby "yield[ing] the desired shape." Vacanti '610 at col. 3, lns. 65-66. The exemplary applications of such struts are "heart valve 'leaflets' and tubes." *Id.* at col. 3, lns. 66-67. The single paragraph in which the struts are disclosed in detail is reproduced below:

> In some embodiments it may be desirable to create additional structure using devices provided for support, referred to herein as "struts." These can be biodegradable or non-degradable polymers which are inserted to form a more defined shape than is obtained using the cell-matrices. An analogy can be made to a corset, with the struts acting as "stays" to push the surrounding tissue and skin up and away from the implanted cells. In a preferred embodiment, the struts are implanted prior to or at the time of implantation of the cell-matrix structure. The struts are formed of a polymeric material of the same type as can be used to form the matrix, as listed above, having sufficient strength to resist the necessary mechanical forces.

The structural relationship between the struts and the cell-matrix structure is not specifically disclosed in this paragraph. Nevertheless, it seems clear that the struts are separate from the cell-matrix structure. There is no indication that the struts are to be part of a matrix onto which cells

are seeded ex vivo, as required by the present claims. Rather, the struts are described as a separate structural element, which may be implanted either simultaneously with or prior to the implantation of the cell-matrix structure. The struts are said to function in the manner of corset stays, exerting force on the tissue and skin surrounding an implanted graft to push it "up and away from the implanted cells" (emphasis added). These disclosures strongly suggest that rather than being "integrated with the sponge", as required by pending Claim 7, the struts are entirely separate from the cell-matrix structure. Thus, the only suggestion in this reference is to use a matrix unlike that presently claimed.

Moreover, there is also no disclosure or suggestion in Vacanti that the struts should be located inside the matrix or on the exterior surface thereof prior to seeding the cells thereon, as required by pending Claim 7. If the struts were located within the cell-matrix structure, for example, they would be unable to perform the disclosed function of "push[ing] the surrounding tissue and skin up and away from the implanted cells." Rather, at least some of the cells would be forced against the surrounding tissue by action of the internal struts. Similarly, struts that were integral with and on the exterior surface of the cell-matrix structure would leave the implanted cells at the surface of the matrix in contact with the surrounding tissue or skin, which is inconsistent with the disclosed function of pushing the tissue or skin "away from" the matrix. It is unclear why the Examiner characterizes this function as not "critical," Office Action at 8. It is the only concrete disclosure indicating how the struts provide support to the engineered graft. Because modifying the Vacanti disclosure to place the struts inside or on the exterior surface of the matrix would render them incapable of carrying out the tissue pushing function disclosed, one of skill in the art would have no reason to modify the Vacanti disclosure in this way.

With respect to the examples cited by the Examiner at pages 4-5 of the Office Action, Applicant notes that neither example discloses the use of the "struts" that the Examiner equates with the claimed reinforcements. In Example 2, cardiovascular tissue (a blood vessel, as in pending Claim 8) was engineered, and the extracellular matrices of the resulting tissue were examined to determine if the engineered tissue had "the physical characteristics of native vascular tissues." Vacanti '610 at col. 9, lns. 7-11. The results showed that vascular tissue had

been "successfully formed" without the use of reinforcements. *Id.* at lns. 13-14. Moreover, the engineered heart valve (as in pending Claim 9) of Example 1 was actually implanted into a sheep "to determine if the constructs had the required pliability and mechanical strength for use in patients." Vacanti '610 at col. 8, lns. 21-23. The results of the implantation are not given in the Vacanti reference. From this inclusion of this working example in the patent specification, however, one of skill in the art would assume that the unreinforced engineered heart valve performed adequately. In other words, Vacanti '610 discloses the engineering of unreinforced cardiovascular tissues that were found to have the physical characteristics of native tissue, and were actually tested *in vivo* and found to have the "required pliability and mechanical strength" for use in human beings. This testing would indicate to one of skill in the art that engineered cardiovascular tissue needed no reinforcement at all, let alone the specific claimed reinforcement that is integral with the sponge of the matrix and inside or on the exterior surface thereof, as required by amended Claim 7. Vacanti '610 thus indicates (as does Naughton '531, as discussed below) that the replacement tissues grown on the disclosed unreinforced matrix have sufficient strength to be implanted in the body as prosthetic cardiovascular tissue once the tissue culture is complete. One of skill in the art would simply not be motivated to modify the cell matrices of Vacanti '610 in the manner set forth in amended Claim 7. As such, the Vacanti '610 reference fails to support a *prima facie* showing of obviousness.

Furthermore, even had a *prima facie* showing of obviousness been set forth, the presently claimed invention provides unexpected advantages that would effectively rebut such a showing. The integration of the claimed reinforcement with the sponge and locating it either inside or on the exterior surface of the matrix, as required by amended Claim 7, provides the unexpected advantages that a smooth sponge surface comes into contact with the blood flow, which is advantageous in that it reduces the likelihood that thromboses will be formed, enhances cell adhesion, promotes the smooth supply of nutrition to the cells, and enhances the formation of the tunica intima. These effects of the present invention could not have been expected from Vacanti '610 by a person skilled in the art. Accordingly, Vacanti '610 neither discloses nor suggests to

one of skill in the art the use of a reinforcement which is integrated with the sponge and located inside or on the exterior surface of the matrix, as required by amended Claim 7.

b. Vyakarnam's Reinforcements Are Not Disclosed to be Inside or on the Exterior Surface of the Matrix

Vyakarnam '084 discloses foam structures that can be composed of copolymers of lactide and which can be used to regenerate tissue, such as vascular grafts. As the Examiner notes, Vyakarnam teaches that the foam may be reinforced with fibers made of calcium phosphate. However, Vyakarnam does not disclose the structural relationship between the reinforcing fibers and the foam. The only discussion of these reinforcements is quoted below:

> [A]t the bottom of this structure there is a need for larger pores (about 150 μm to about 300 μm) with higher stiffness to be structurally compatible with cancellous bone. The foam in this section could be reinforced with ceramic particles or fibers made up of calcium phosphates and the like.

Vyakarnam '084 at col. 6, lines 36-41 (emphasis added). The Examiner presumes that this disclosure meets the limitation, but there is no indication here of the structural relationship between the reinforcing fibers and the foam. The fibers may be implanted separately from an engineered graft, as are Vacanti's struts. They may be on the inside surface of the foam. Vyakarnam is simply silent on this point, and the Examiner is not entitled to fill this gap in the Vyakarnam disclosure with Applicants' own claim limitation.

Furthermore, the Examiner points to this disclosure as an example of tissue engineering. This appears to be a misunderstanding of the passage. Vyakarnam is concerned in this section with the "stiffness... at the time of implantation" and that the layers of the matrix when implanted "be able to support the environmental loading and thereby protect the invading cells until they have differentiated and consolidated into tissue that is capable of sustaining load." Vyakarnam at col. 6, lines 17-18, 20-23 (emphasis added). Therefore the Examiner's assumption that this disclosure relates to tissue engineering methods such as those claimed in the present application is unwarranted, and there would be no apparent reason to modify the matrix used for cardiovascular tissue engineering in a similar way.

Furthermore, there is no reason for one of skill in the art to modify Vyakarnam's disclosure in the particular way set forth in Claim 7. The discussion of fibers to which the Examiner refers is within a section of the Vyakarnam specification discussing cartilage. Here, however, the reinforcing fibers are restricted to a section of the cartilage that attaches to bone, in which "higher stiffness" is required "to be structurally compatible with cancellous bone." Id. (emphasis added). Furthermore, Vyakarnam also discusses the use of fibers in tissue scaffoldings for bone repair, which would clearly also require significant stiffness. Vyakarnam '084 at col. 8, lines 5-12. But Vyakarnam does not disclose the use of such fibrous reinforcements in tissue scaffoldings for use in vascular repair. One of skill in the art would accordingly understand that the use of reinforcing fibers is only preferable where higher stiffness is required, such as in those parts of cartilage which are connected to bone or in bone itself, and that such reinforcement should not be used for cardiovascular tissue. Much less does Vyakarnam suggest to one of skill in the art the use of the specific claimed reinforcement of amended Claim 7, which is integrated with the sponge and located inside or on the exterior surface of the matrix.

In response to Applicants' arguments on this point, the Examiner asserts that stiffness provides strength, and cites as evidence Applicants' unreinforced matrix, which in his view is "less stiff than the sponge containing the plain-weave cloth." Office Action at 9-10. It is unclear on what basis the Examiner concludes that inclusion of a "cloth" would add any stiffness to the material. Even if it did, stiffness does not necessarily provide strength. For example, a thin strip of wood can be highly stiff, but not particularly strong. Furthermore, the Examiner forgets that cardiovascular tissue is required to be not only strong but pliable. See Vacanti '610 at col. 8, line 22 (describing testing of the engineered heart valves for the "required pliability" for use in patients). One of skill in the art of engineering vascular grafts would not understand from Vyakarnam's disclosure that it would be appropriate to add stiffness to the engineered cardiovascular tissue in order to provide strength. Such a person would, rather, conclude that Vyakarnam's reinforcements would add undesirable stiffness, which would probably render the cardiovascular tissue graft unsuitable for its intended purpose. This would lead one of skill in the art away from the cited combination. See In re Sponnoble, 405 F.2d 578, 587 (C.C.P.A.

1969) (finding no reason to combine references that, if combined, "would produce a seemingly inoperative device"); see also Tec Air, Inc. v. Denso Mfg. Mich. Inc., 192 F.3d 1353, 1360 (Fed. Cir. 1999) ("If when combined, the references 'would produce a seemingly inoperative device,' then they teach away from their combination." (quoting Sponnoble, 405 F.2d at 587)).

For these reasons, Vyakarnam '084 neither discloses nor suggests to one of skill in the art the use of a reinforcement which is integrated with the sponge and located inside or on the exterior surface of the matrix, as required by amended Claim 7.

c. One of Skill in the Art Would Have No Reason to Combine Morita With Vacanti or Vyakarnam

The sole remaining source for a disclosure corresponding to the "integrated with the sponge and located inside or on the exterior surface of the matrix" limitation available to the Examiner is Morita '864. Morita discloses a filler material for use *in vivo* that uses poly-L-lactic acid. Morita does not disclose seeding and growing cells on the filler material prior to implantation. Neither does Morita disclose that the filler material is used for regenerating blood vessels or similar cardiovascular tissue structures.

Because Morita does not teach ex vivo seeding of cells onto the open-cell foam or sponge that is to be implanted, it is not surprising that he should have been concerned with strengthening the porous implant before subjecting it to the stresses present *in vivo*. Morita suggests that this reinforcement is not necessary when fully regenerated tissue is present. Specifically, the implant disclosed in Morita is said to "maintain[] its strength and shape over a long period of time until the regeneration of the tissue." Morita translation at page 5, lines 13-14 (Morita '864 at page 7, lines 5-7). By implication, regenerated tissue would not require the bioabsorbable reinforcement disclosed in Morita. Therefore, in contrast to the Examiner's understanding, Morita suggests that reinforcement would not be required when tissue is regenerated before implantation.

In short, one of skill in the art, reading Morita before the claimed invention was made, would have understood him to teach that replacement tissues grown on the disclosed matrices or foams without a bioabsorbable reinforcement have sufficient strength to be implanted in the body as prosthetic cardiovascular tissue once the tissue culture is complete, such that the inclusion of a

bioabsorbable reinforcement is not required. Certainly, Morita would have not raised any doubts about the resistance to rupture of engineered cardiovascular tissue grown on unreinforced materials, because Morita contained no disclosure relating to tissue engineering. There would accordingly be no reason for one of skill in the art to combine Morita's reinforcement with the unreinforced materials of the other references.

The Examiner states that, in Morita, "the sponge used to produce the regenerated tissue is reinforced with fibers to maintain strength and shape during production of the tissue, and after the tissue has been regenerated the sponge has been absorbed into the body." Office Action at 10. This may well be true, but it is unclear how it bears on the question of whether one of skill in the art would see a reason to combine the disclosures. One of skill in the tissue engineering art would understand that greater stresses are present *in vivo* than *in vitro*, and that without the additional strength of the cultured network of cells, an implant would require additional reinforcement to resist those stresses.

The Examiner has the burden under Section 103 to establish a *prima facie* case of obviousness. In re Fine, 837 F.2d 1071, 1074 (Fed. Cir. 1988). The Examiner has not shown that there is any reason for one of skill in the art to combine the cited references, and has thus not met this burden. The Examiner's rejection of these claims as obvious under 35 U.S.C. § 103(a) is thus legally deficient and should be reversed.

3. The Unexpected Results Demonstrated by the Applicants are Objective Evidence of Nonobviousness That the Examiner Improperly Discounted

In evaluating whether a claimed invention is obvious under Section 103, the Examiner is also required to consider whether unexpected results were obtained: "objective evidence o[f] secondary considerations such as unexpected results ... are relevant to the issue of obviousness and must be considered in every case in which they are present." M.P.E.P. § 2141. Furthermore, all available evidence, including evidence of secondary considerations, must be considered in the course of reaching the conclusion of obviousness. In re Fine, 837 F.2d at 1073-74 ("'To reach a proper conclusion under § 103, the decisionmaker must step backward in time and into the shoes worn by [a person having ordinary skill in the art] when the invention was unknown and just

before it was made. In light of *all* the evidence, the decisionmaker must then determine whether ... the claimed invention as a whole would have been obvious at *that* time to *that* person.'" (quoting Panduit Corp. v. Dennison Mfg. Co., 810 F.2d 1561, 1566 (Fed. Cir. 1987))).

In this case, as noted above, Applicants demonstrated that an engineered blood vessel graft that was not provided with a bioabsorbable reinforcement, as suggested by the prior art, failed within one week when it was grafted into the vena cava of a dog. In contrast, a cardiovascular graft that was produced using a bioabsorbable reinforcement in accordance with the present disclosure did not rupture, showed good patency on angiographic examination three months after implantation, and after six months *in vivo* the blood vessel had regenerated at the implantation site. See specification at pages 12-13. The Examiner does not seem to have recognized these as unexpected results, however, and simply states that: "it would have been obvious to provide reinforcement as suggested by Vacanti et al, Vyakarnam et al and the Japanese patent." Office Action at 9.

Objective evidence of secondary considerations such as unexpected effects must, of course, be considered before determining whether an invention is obvious over the prior art. See M.P.E.P. § 2141; In re Vamco Machine & Tool, Inc., 752 F.2d 1564, 1573 (Fed. Cir. 1985) ("'[E]vidence rising out of the so-called 'secondary considerations' must always when present be considered en route to a determination of obviousness.'" (quoting Stratoflex, Inc. v. Aeroquip Corp., 713 F.2d 1530, 1538 (Fed. Cir. 1983))). Here, the Examiner appears to have come to the conclusion that the invention was obvious ("it would have been obvious to provide reinforcement") and rejected the evidence of unexpected effects on the basis of that conclusion (as well as his unsupported conclusion that the Vacanti material was stronger), rather than considering the evidence before concluding that the claims were obvious.

In this case, if bioabsorbable reinforcements were in fact needed in cardiovascular grafts, the prior art would have taught or suggested that such bioabsorbable reinforcements should be used. The fact that it did not disclose or suggest the use of such bioabsorbable reinforcements is evidence that the use of such bioabsorbable reinforcements is not obvious.

The results obtained by the applicants demonstrate that the blood vessel grafts produced in accordance with the pending claims were unexpectedly superior to those produced by methods suggested by the prior art. This objective evidence of unexpected effects is a strong indicator that the pending claims are not obvious in view of the cited prior art.

The rejection of the pending claims as obvious under 35 U.S.C. § 103(a) should be reversed on this ground.

B. Claim 10 Is Not Obvious Over the Combination of Vacanti '610 and Fofonoff et al., United States Patent No. 5,882,929, taken with Cox, United States Patent No. 6,719,789, or Love, United States Patent No. 5,509,930

The Examiner has rejected Claim 10 over the combination of Vacanti '610 with Fofonoff et al., U.S. Patent No. 5,882,929, taken with Cox, U.S. Patent No. 6,719,789, or Love, U.S. Patent No. 5,509,930. Claim 10 depends from Claim 7, and incorporates all the limitations thereof. As described above, Vacanti '610 does not disclose or suggest a reinforcement that is integrated with the sponge and located inside or on the exterior surface of the matrix, as required by Claim 7. Fofonoff '929, Cox '789, and Love '930 do not disclose or suggest the use of reinforcements in the production of cardiovascular tissue, let alone the use of the specific claimed reinforcements. Accordingly, they cannot supply the disclosure lacking in Vacanti '610. For that reason, Claim 10 is not obvious over this combination of references.

C. Claims 7, 8, and 11 Are Not Obvious Over Naughton, et al., United States Patent No. 5,863,531

The Examiner has rejected Claims 7, 8, and 11 as obvious over Naughton et al., U.S. Patent No. 5,863,531. As amended, Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." Naughton '531 neither discloses nor suggests such a reinforcement.

During prosecution, claim scope is determined "not solely on the basis of the claim language, but upon giving claims their broadest reasonable construction 'in light of the specification as it would be interpreted by one of ordinary skill in the art.'" Phillips v. AWH Corp., 415 F.3d 1303, 1316 (Fed. Cir. 2005) (en banc) (quoting In re Am. Acad. Of Sci. Tech Ctr., 367 F.3d 1359, 1364 (Fed. Cir. 2004)). In this case, one of skill in the art would understand

from the specification that the claimed reinforcement is an artificial fiber, nonwoven fabric or film that is present in the sponge before any cells are seeded thereon. See specification at 6, 8. The reinforcement is nowhere described in terms of a matrix produced by a particular cell type seeded onto the sponge. Indeed, the very same type of cells that Naughton employs to produce the elastin-containing matrix—fibroblasts—are also seeded onto the sponge in Applicants' comparative example. See specification at 12. The subsequent failure of the engineered graft would indicate to one of skill in the art that the matrix produced by the seeded fibroblasts could not be the claimed "reinforcement," because the resulting graft failed. The Examiner's reading of the "reinforcement" limitation of Claim 7 so as to embrace an "extracellular matrix containing elastin produced during the culturing of stromal cells," Office Action at 13, is therefore unreasonably broad in light of the specification.

Properly understood, Naughton simply suggests that tubular biological replacement tissues grown on an unreinforced matrix may successfully be used as cardiovascular replacement tissues *in vivo*. The examples of tubular biological tissue engineering provided all use unreinforced mesh as the starting point. *See* Naughton '531 at col. 23. Furthermore, Naughton discloses that arterial structures grown in this way produce elastin, so as to "simulate . . . natural arterial walls." Naughton '531 at col. 25, lns. 9-10. Naughton would accordingly suggest to one of skill in the art that the replacement tissues grown on unreinforced matrices or foams have sufficient strength to be implanted in the body as prosthetic cardiovascular tissue once the tissue culture is complete. Naughton thus did not know of the problem of graft failure associated with unreinforced matrices used to regenerate cardiovascular tissue, nor did he suggest reinforcement of such matrices.

Much less does Naughton disclose or suggest the specific reinforcement required by amended Claim 7, which is integrated with the sponge and located inside or on the exterior surface of the matrix. Because Naughton '531 neither discloses nor suggests such a reinforcement, Claim 7, and Claims 8 and 11, which depend therefrom, are not obvious over Naughton '531.

D. Claims 9, 15, and 19 Are Not Obvious Over the Combination of Naughton '531 and Vacanti '610

The Examiner has rejected Claims 9, 15, and 19 as obvious over the combination of Naughton '531 and Vacanti '610. Claims 9, 15, and 19 all depend from Claim 7, and incorporate all the limitations thereof. Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." As described above, neither Naughton '531 nor Vacanti '610 discloses or suggests the use of a matrix comprising such a reinforcement in engineering cardiovascular tissue grafts. Accordingly, Claims 9, 15, and 19 are not obvious over the combination of Naughton '531 and Vacanti '610.

E. Claim 10 Is Not Obvious Over the Combination of Naughton '531, Vacanti '610, and Fofonoff '929, Taken With Cox '789 or Love '930

The Examiner has rejected Claim 10 as obvious over the combination of Naughton '531 and Vacanti '610, and further in view of Fofonoff '929, Cox '789, and Love '930. Claim 10 depends from Claim 7, and incorporates all the limitations thereof. Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." As described above, none of Naughton '531, Vacanti '610, Fofonoff '929, Cox '789, or Love '930 discloses or suggests the use of a matrix comprising such a reinforcement in engineering cardiovascular tissue grafts. Accordingly, Claim 10 is not obvious over this combination of references.

F. Claims 16-18 Are Not Obvious Over the Combination of Naughton '531, Vacanti '610, Vyakarnam '084, and Morita '864

The Examiner has rejected Claims 16-18 as obvious over the combination of Naughton '531, Vacanti '610, Vyakarnam '084, and Morita '864. Claims 16-18 all depend from Claim 7, and incorporate all the limitations thereof. Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." As described above, none of Naughton '531, Vacanti '610, Vyakarnam '084, or Morita '864 disclose or suggest the use of a matrix comprising such a reinforcement in engineering cardiovascular tissue grafts. Accordingly, Claims 16-18 are not obvious over this combination of references.

G. Claims 7-11 and 15-19 Are Not Indefinite Under 35 U.S.C. § 112

Finally, the Examiner has rejected all of the pending claims under 35 U.S.C. § 112 as indefinite. The Examiner believes the meaning of "reinforcement being integrated with the

sponge" to be "uncertain as to meaning and scope," because "the specification fails to define the difference between being integrated and not integrated with the sponge." Office Action at 2. In effect, the Examiner penalizes Applicants for choosing to rely on the ordinary meaning of "integrated" rather than act as their own lexicographers. Surely there is no need for the Applicants to separately define such a well-understood term. Webster's, for example, defines the word as "combining or coordinating separate elements so as to provide a harmonious, interrelated whole." Webster's Encyclopedic Unabridged Dictionary of the English Language 738. An example thereof is shown in Figure 1 of the present application, which depicts the reinforced matrix prior to the seeding of cells thereon. Vacanti's struts, in contrast, which may be implanted before the seeded matrix, are clearly separate from the cell matrix and thus cannot be "integrated" with it. The "reinforcement being integrated with the sponge" limitation is not indefinite and the rejection of the pending claims under 35 U.S.C. § 112 should be reversed.

CONCLUSION

In view of the arguments presented above, appellants submit that the pending claims are not obvious in view of the cited prior art combination and respectfully request that the Section 103(a) obviousness rejection be reversed, that the Section 112 indefiniteness rejection be reversed, and that the application be allowed.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 7 SEPTEMBER 2007

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VIII. CLAIMS APPENDIX

1-6. (Cancelled)

7. (Previously presented) A method for regenerating cardiovascular tissue comprising:

seeding cells on a matrix comprising a sponge configured to regenerate cardiovascular tissue and made of a bioabsorbable material and a reinforcement made of a bioabsorbable material, the reinforcement being integrated with the sponge and located inside or on the exterior surface of the matrix;

culturing the cells until the matrix surface is completely covered with the cells; and

embedding the matrix in vivo for regenerating cardiovascular tissue.

8. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a blood vessel.

9. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a cardiac valve.

10. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a pericardium.

11. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cells to be seeded are a mixed cell culture of two or three different kinds selected from the group consisting of endothelial cells, smooth muscle cells and fibroblasts.

12-14. (Cancelled)

15. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7, wherein the bioabsorbable material is at least one member selected from the group consisting of polyglycolic acid, polylactic acid (D form, L form, or DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form) copolymers,

glycolic acid-caprolactone copolymers, lactic acid (D form, L form, DL form)-caprolactone copolymers and poly(p-dioxanone).

16. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating an artery, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

17. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating a vein, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polyglycolic acid.

18. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating a cardiac valve or a pericardium, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

19. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7, wherein the sponge has a pore diameter of about 5 μm to about 100 μm.

IX. EVIDENCE APPENDIX

1. Specification and figures as filed;
2. Office Action mailed September 8, 2004;
3. Response to Office Action, filed February 8, 2005;
4. Office Action mailed May 17, 2005;
5. Response to Office Action, filed September 19, 2005;
6. Advisory Action mailed October 12, 2005;
7. Office Action mailed June 1, 2006;
8. Response to Office Action, filed December 1, 2006;
9. Office Action mailed March 12, 2007;
10. United States Patent No. 5,855,610;
11. United States Patent No. 6,534,084;
12. United States Patent No. 5,863,531;
13. Japanese Patent No. 03-23864 and English translation;
14. United States Patent No. 5,882,929;
15. United States Patent No. 6,719,789;
16. United States Patent No. 5,509,930.

X. RELATED PROCEEDINGS APPENDIX

There are no decisions rendered by a court or the Board in any related proceedings identified above.

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DESCRIPTION

MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND METHOD FOR REGENERATING CARDIOVASCULAR TISSUE

TECHNICAL FIELD

The present invention relates to a matrix for culturing cardiovascular cells to regenerate cardiovascular tissue and a method for regenerating cardiovascular tissue such as an artificial blood vessel, cardiac valve, pericardium, etc.

BACKGROUND ART

In the field of artificial vessels, for instance, those made of non-bioabsorbable polymers are widely used. An artificial vessel (GORE-TEX), for example, is used most frequently in a clinical field. Such non-bioabsorbable artificial vessel is excellent in physical properties; however, because of the non-bioabsorbability, it remains in vivo as a foreign body for a long period of time after implantation. Further, when the non-bioabsorbable artificial vessel is implanted into the body of a child, another surgery for replacement is necessary since the non-bioabsorbable artificial vessel does not expand with the growth of the autogeneous blood vessel.

A tissue regeneration method employing tissue engineering techniques has recently been developed, wherein cells of autogeneous tissue are seeded and

cultured on a scaffold made of a bioabsorbable polymer so as to regenerate the autogeneous tissue. There have been published quite a few research reports of the tissue regeneration method applied to skin regeneration (M. L. Cooper, L. F. Hansbrough, R. L. Spielvogel, et al.: In vivo optimization of a living dermal substitute employing cultured human fibroblasts on a biodegradable polyglycolic acid or polyglactin mesh. Biomaterials, 12:243-248, 1991) and cartilage regeneration (C.A. Vacanti, R. Langer, et al.: Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. Plast. Reconstr. Surg., 88:753-759, 1991).

If a blood vessel can be regenerated in the same manner as described above, growth of the regenerated blood vessel is expected since it is regenerated by using autogeneous tissue and no longer necessitates the use of anti-coagulants.

An object of the present invention is to provide a matrix which allows cells to sufficiently adhere thereto, provides an optimum scaffold for cell proliferation, maintains satisfactory blood flow resistance in vivo till autogeneous tissue is regenerated, and is ultimately decomposed and absorbed in vivo.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a photograph showing a cross-sectional

view of a vascular regeneration matrix according to the present invention.

Fig. 2 is a photograph showing a plan view of a vascular regeneration matrix according to the present invention.

Fig. 3 is a photograph of the angiogram recorded at the 3rd postoperative month.

DISCLOSURE OF INVENTION

Basic requirements for the matrix for culturing cardiovascular cells to regenerate cardiovascular tissue are an ability to allow cells seeded thereon to adhere firmly thereon and a bioabsorbability which enables the matrix to be absorbed in vivo when a blood vessel is regenerated. A sponge is considered to be the optimum material to fulfill the above requirements.

In the case of using the matrix for regenerating a blood vessel, the matrix is required to maintain an enough strength to endure a blood flow for a certain period of time after implantation till the blood vessel is regenerated in vivo.

The inventors found that the above object is achieved by strengthening, with a reinforcement made of a bioabsorbable material, a sponge made of a bioabsorbable material which is an optimum scaffold for cell proliferation and excellent in cell adhesiveness.

The present invention provides a matrix for culturing cardiovascular tissue and a method for regenerating cardiovascular tissue of the following items.

Item 1. A matrix for culturing cardiovascular cells to regenerate cardiovascular tissue comprising a sponge made of a bioabsorbable material and a reinforcement made of a bioabsorbable material.

Item 2. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1, wherein the bioabsorbable material is at least one member selected from the group consisting of polyglycolic acid, polylactic acid (D form, L form, DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form) copolymer, glycolic acid-caprolactone copolymer, lactic acid (D form, L form, DL form)-caprolactone copolymer, poly(p-dioxanone) and the like.

Item 3. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1 for use in regenerating an artery, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

Item 4. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1 for use in regenerating a vein, wherein the sponge comprises a lactic acid-caprolactone copolymer and the

reinforcement comprises polyglycolic acid.

Item 5. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1 for use in regenerating a cardiac valve or a pericardium, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

Item 6. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to item 1, wherein the sponge has a pore diameter of about 5 μm to about 100 μm.

Item 7. A method for regenerating cardiovascular tissue comprising seeding cells on the matrix of item 1 and culturing the cells.

Item 8. The method for regenerating cardiovascular tissue according to item 7, wherein the cardiovascular tissue to be regenerated is a blood vessel.

Item 9. The method for regenerating cardiovascular tissue according to item 7, wherein the cardiovascular tissue to be regenerated is a cardiac valve.

Item 10. The method for regenerating cardiovascular tissue according to item 7, wherein the cardiovascular tissue to be regenerated is a pericardium.

Item 11. The method for regenerating cardiovascular tissue according to item 7, wherein the

cells to be seeded are a mixed cell culture of two or three different kinds selected from the group consisting of endothelial cells, smooth muscle cells and fibroblasts.

According to the invention, it is preferable that regeneration of cardiovascular tissue be conducted by seeding cells to a matrix for culturing cardiovascular cells and embedding the matrix in vivo to regenerate cardiovascular tissues in vivo.

Examples of bioabsorbable material include polyglycolic acid, polylactic acid (D form, L form, DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form) copolymer, glycolic acid-caprolactone copolymer, lactic acid (D form, L form, DL form)-caprolactone copolymer, poly(p-dioxanone) and the like.

Examples of cardiovascular tissue include blood vessels, cardiac valves, the pericardium and the like.

The matrix of the invention is obtained by strengthening a sponge made of a bioabsorbable material with a reinforcement (in the form of a fiber, nonwoven fabric or film) made of a bioabsorbable material. There is no limitation on the bio-absorbable materials to be used for the sponge and the reinforcement. In the case of preparing the matrix for regenerating a blood vessel, a sponge made of a lactic acid-caprolactone copolymer may be combined with a reinforcement made of polylactic acid when

the blood vessel is an artery, and the same sponge may be combined with a reinforcement made of polyglycolic acid when the blood vessel is a vein. Further, in the case of regenerating a cardiac valve or the pericardium, a sponge made of a lactic acid-caprolactone copolymer may be combined with a reinforcement made of polylactic acid.

The sponge has pores each having such a pore size that cells can suitably be adhered thereto to proliferate and that no blood leakage is caused when the matrix comprising the sponge is implanted as cardiovascular tissue. The pore size may typically be about 1 mm or less, preferably about 5-100 μm. The shape of the matrix may be cylindrical when the cardiovascular tissue to be regenerated is a blood vessel or may be plane when the cardiovascular tissue to be regenerated is a cardiac valve or the pericardium. In the case of regenerating a blood vessel, the length and inside diameter of the matrix may be adjusted depending on the target blood vessel. The thickness of the matrix is chosen depending on the desired period for bio-absorption or ease of suturing. The thickness may typically be about 5 mm or less, preferably from about 500 μm to about 2 mm.

For preparation of the sponge, the following alternative processes, among others, are available.

(1) Lyophilization process

A substrate polymer solution is poured in a mold, frozen, and, then lyophilized. According to the freezing temperature and polymer concentration, sponges having various pore diameters are obtained (described in Examples).

(2) Elution process

A water-soluble material is mixed with the substrate polymer solution and, after drying, the water-soluble material is washed out with rinse water. The resultant sponge has a pore diameter corresponding to the particle size of the water-soluble material used. In the present case, sucrose can be used with advantage.

The reinforcement must have a greater strength than the sponge. The reinforcement can be selected from among a fiber, nonwoven cloth, film and so on.

The reinforcement is preferably integrated with the sponge and can be located either on the interior surface, inside, or exterior surface of the sponge. However, since the interior surface of the sponge is involved in the adhesion of vascular endothelial cells, it is preferably situated inside or on the exterior surface, although the interior surface may be optionally used.

As to the cells to be seeded, substantially the same kinds of cells are used for various cardiovascular tissues in common. Thus, they are endothelial cells,

smooth muscle cells and fibroblasts, and a mixed cell culture of two or three different kinds of cells can be mentioned by way of example. Tissue construction is carried out using such mixed culture cells.

The cultural conditions for the cells to be used and the seeding method are described below.

A. Cell isolation, culture, and propagation

The vascular tissue isolated in a sterile environment is immersed in a cell culture medium and washed with phosphate-buffered saline in a clean bench. Then, on a Petri dish, the tissue is cut into pieces using a surgical knife according to the simple explant technique. Tissue pieces sized about 1-2 mm^2 are distributed uniformly on the dish and after about 20 minutes, when the tissue pieces have intimately adhered to the bottom of the dish, a culture medium is added. As the culture medium, Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10 % fetal calf serum and 1 % antibiotics solution (L-glutamine 29.2 mg/ml, penicillin G 1000 U/ml, streptomycin sulfate 10,000 µg/ml) is used. The mixed cells of endothelial cells and fibroblasts begin to migrate from the tissue pieces on the dish after 5-7 days, forming mixed-cell colonies around the explants in a further one week. After another 2-3 weeks, the mixed cells become confluent on the dish. Immediately, a passage is made

using 0.25 % trypsin and the culture in a 75 cm^2 culture flask is started. Generally when the growth in this flask has become confluent, about $2x10^6$ cells are available. Cell culture is performed under an atmosphere comprising 5 % CO_2 and 21 % O_2 and continued until $10x10^6$ cells have been obtained. While the culture medium is renewed every 4-5 days, the resultant of a preliminary experiment has shown that the doubling time of cells is about 48 hours. Incidentally, the counting of cell population during the course is carried out by the classical exclusion method using Trypan Blue.

B. Cell sorting and endothelial cell purification

At the stage when the mixed cells have become confluent and a reasonable number of cells is obtained, endothelial cells are sorted out from among the mixed cells using FACS according to the following protocol. Thus, Dil-acetylated LDL (fluorescent dye marker; product of Biomedical Technologies) (briefly, Dil-Ac-LDL) is added to the mixed cell culture at a concentration of 1 μg/ml, followed by 24-hour incubation. This marker is taken up intracellularly through a scavenger pathway specific to endothelial cells and macrophages. After 24 hours, the cells are trypsinized to prepare a mixed cell suspension and sorting is performed using a cell sorter (FACS machine; product of Bectin Dickenson). According to the

size and emission of fluorescence, the cells are sorted into Dil-Ac-LDL-positive cells and Dil-Ac-LDL-negative cells. After the sorting, these types of cells are independently cultured and the culture is continued until $2x10^6$ endothelial cells are obtained.

C. Tissue construction

The first step of tissue construction comprises seeding cells in vitro. Specifically, a biodegradable culture matrix is seeded with about $1x10^6$ cells/cm^2 of Dil-Ac-LDL-negative fibroblasts.

Immediately following the seeding of a concentrated cell suspension on the matrix, the system is allowed to stand on the culture dish in a clean bench for 30-60 minutes, and thereafter about 50 ml of a culture medium is added. The culture medium is renewed every day as a rule and after 7 days, that is, one day before surgical transplantation, a further seeding is performed with a suspension of endothelial cells (about $2x10^6$ cells), whereby a monolayer of endothelial cells is obtained.

The above steps A-C show the cell isolation, culture and seeding procedures for the construction of a heart valve, a pericardium, or a blood vessel.

BEST MODE FOR CARRYING OUT THE INVENTION

The following examples are further illustrative of the present invention.

Example 1

· Construction of a vascular regeneration matrix

A glass test tube (10 mm in outside diameter) was wrapped around with a plain-weave cloth of poly-L-lactide fiber (photograph) in a double-cylindrical form. This assembly was set in a mold (12 mm in inside diameter) and a solution of L-lactide-caprolactone copolymer (50:50) in dioxane (5 %) was poured into the clearance, frozen and then lyophilized.

The cylindrical vascular prosthesis thus obtained was a cellular substrate reinforced with a fibrous material (Figs. 1 and 2).

· Cell culture

Through a small skin incision, a peripheral vein segment, about 5 mm long, was excised in a sterile environment and immediately immersed in the tissue culture medium. Cell isolation was carried out by the simple explant technique. As the cell culture medium, the standard cell culture medium DMEM mentioned above was used, and the medium was renewed every 2-3 days.

· Seeding of cells

The matrix prepared above was seeded with about $1x10^6$ cells/cm^2 of a mixed culture of endothelial cells and fibroblasts and the culture was continued for about 1 week until the matrix surface had been completely covered

with the cells.

· Animal experiment

The vascular prosthesis constructed as above was transplanted in the inferior vena cava of a young dog. As a result, no obliteration by rupture was found and a good patency could be verified angiographically at the 3rd postoperative month (the angiograph in Fig. 3). Thoracotomy at 6 months revealed regeneration of the autogenous blood vessel in agreement with the transplantation site.

In contrast, the matrix not reinforced with poly-L-lactide fiber ruptured in one week after substitution and the experimental animal succumbed to sudden death.

Claims

1. A matrix for culturing cardiovascular cells to regenerate cardiovascular tissue comprising a sponge made of a bioabsorbable material and a reinforcement made of a bioabsorbable material.

2. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1, wherein the bioabsorbable material is at least one member selected from the group consisting of polyglycolic acid, polylactic acid (D form, L form, DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form) copolymer, glycolic acid-caprolactone copolymer, lactic acid (D form, L form, DL form)-caprolactone copolymer and poly(p-dioxanone).

3. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating an artery, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

4. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating a vein, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polyglycolic acid.

5. The matrix for culturing cardiovascular

cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating a cardiac valve or a pericardium, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

6. The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1, wherein the sponge has a pore diameter of about 5 μm to about 100 μm.

7. A method for regenerating cardiovascular tissue comprising seeding cells on the matrix of Claim 1 and culturing the cells.

8. The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a blood vessel.

9. The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a cardiac valve.

10. The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a pericardium.

11. The method for regenerating cardiovascular tissue according to Claim 7, wherein the cells to be seeded are a mixed cell culture of two or three different kinds selected from the group consisting of endothelial

cells, smooth muscle cells and fibroblasts.

Abstract

Materials for culturing cardiovascular tissues wherein a sponge made of a bioabsorbable material is reinforced with a reinforcement made of a bioabsorbable material.

FIG. 1

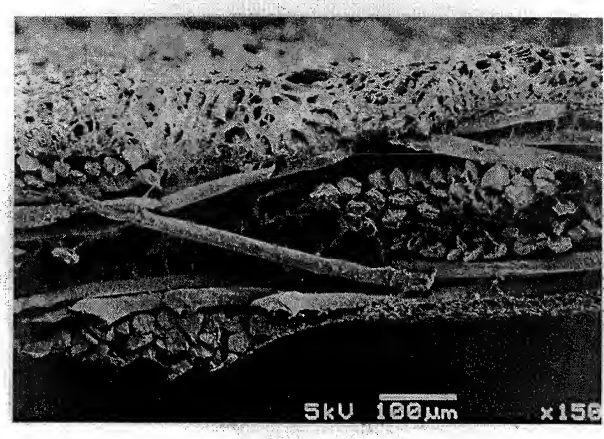

FIG. 2

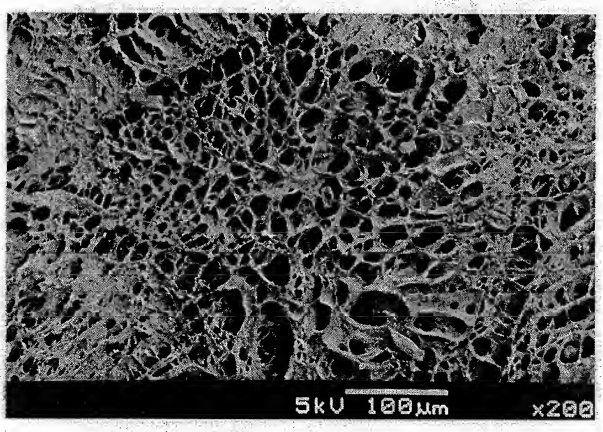

FIG. 3

SAEG108.001APC asg
dea/koa

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/070,938	06/04/2002	Shinichiro Morita	SAEG108.001APC	4758

20995 7590 09/08/2004

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EXAMINER	
NAFF, DAVID M	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 09/08/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/070,938	MORITA ET AL.	
	Examiner	Art Unit	
	David M. Naff	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 June 2002*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-11* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *6/10&9/17/02*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims examined on the merits 1-11 which are all claims in the application.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Naughton et al (5,863,531) in view of Vyakarnam et al (6,534,084 B1) taken with Hinsch et al (EP 0 274 898) (listed on form 1449) and Japanese patent 3-23864 (listed on form 1449).

The claims are drawn to a matrix for culturing cardiovascular cells to regenerate cardiovascular tissue comprising a sponge made of a bioabsorbable material and a reinforcement made of a bioabsorbable material. Also claimed is a method of culturing cardiovascular cells to regenerate cardiovascular tissue by seeding cells on the matrix and culturing the cells. The sponge has a pore diameter of about 5-100 μm.

Naughton et al disclose producing tissue *in vitro* by seeding cells on a three-dimensional structure having interstitial spaces which can be used to form tubular tissue structures (col 6, lines 55-60 and col 22, line 41) such as in the form of blood vessels (col 24, line 33), arteries (col 24, line 37) or veins (col 25, line 24). The three dimensional structure can be made of biodegradable material such as polyglycolic acid, polylactic acid or polyglycolic acid copolymer (col 9, lines 60-62). The three-dimensional structure can be made of a sponge (col 9, line 42).

Vyakarnam et al disclose foam structures that can be composed of copolymers of lactide such as a poly(L) lactide-co-E-caprolactone (col 6, line 45, col 9, lines 53-55 and col 12, lines 5-9), and which can be used to regenerate tissue such as tubular structures such as vascular grafts (col 3, lines 1 and 20-21, and col 9, lines 19-24). The pore size of the foam can be 30-50 μm or 100-200 μm (paragraph bridging cols 4 and 5). The foam can be reinforced with fibers (col 6, line 40).

Hinsch et al disclose a porous implant having a pore size of 10-200 μm for the growth of blood vessels in the form of a foam made of a resorbable polymer such as a copolymer of glycolide and lactide (page 4, lines 1-8). The foam may contain textile reinforcing elements such as fibers or knitted fabrics (page 3, lines 8-13).

The Japanese patent discloses a reinforced collagen sponge for implanting in tissue. The sponge is reinforced with fibers made of poly-L-lactic acid.

It would have been obvious to form the biodegradable polyglycolic acid copolymer tubular structures of Naughton et al with the biodegradable foam of Vyakarnam et al that is a copolymer of glycolide and lactide since this foam can be used for producing a tubular structure and has advantageous properties. It would have been further obvious to reinforce the foam with fibers as suggested by Hinsch et al and the Japanese patent using fibers to reinforce a foam implant. It would have been obvious to use fibers formed of poly-L-lactic acid as taught by the Japanese patent so that both the foam and fibers are bioabsorbable. Using polyglycolic acid as in claim 4 to form the fibers would have been a matter of obvious choice. A foam as disclosed by the references is a sponge. When forming the tubular structures of Naughton et al, cardiovascular cells are used and the tissue produced is cardiovascular tissue. The conditions of dependent claims would have been obvious from the disclosures of the references. Structures other than tubular structures such as a cardiac valve or pericardium as in certain dependent claims would have been obvious

from the many different structures disclosed by Naughton et al. Using two or more different cells as in claim 11 would have been obvious from Naughton et al using different types of cells together.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David M. Naff whose telephone number is 571-272-0920. The examiner can normally be reached on Monday-Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

David M. Naff
Primary Examiner
Art Unit 1651

DMN
9/3/04

Notice of References Cited	Application/Control No. 10/070,938	Applicant(s)/Patent Under Reexamination MORITA ET AL.	
	Examiner David M. Naff	Art Unit 1651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,863,531	01-1999	Naughton et al.	424/93.7
	B	US-6,534,084	03-2003	Vyakarnam et al.	424/443
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. SAEG108.001APC	APPLICATION NO. 10/070,938 PCT APPLICATION NO. PCT/JP00/06129
INFORMATION DISCLOSURE STATEMENT BY APPLICANT	APPLICANT Morita, et al.	
(USE SEVERAL SHEETS IF NECESSARY)	FILED DATE: March 7, 2002 PCT FILING DATE: September 8, 2000	GROUP Unknown

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE (IF APPROPRIATE)

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
		WO 84 84/00302	02/02/84	WIPO				
		EP 0 274 898	07/20/88	Europe				
		EP 0277 678	08/10/88	Europe				
		WO 96/08213	03/21/96	WIPO				
		EP 0 734 736	10/02/96	Europe				
		WO 96/38188	12/05/96	WIPO				
		WO 96/40175	12/19/96	WIPO				
		JP 63-255068	10/21/88	Japan			Abstract	
		JP 63-272355	11/09/88	Japan			Abstract	
		JP 1-230366	09/13/89	Japan			Abstract	
		JP 2-167156	06/27/90	Japan			Abstract	
		JP 3-23864	01/31/91	Japan			Abstract	
		JP 5-76588	03/30/93	Japan			Abstract	
		JP 5-269196	10/19/93	Japan			Abstract	
		JP 6-292716	10/21/94	Japan			Abstract	
		JP 10-234844	09/08/98	Japan			Abstract	

EXAMINER INITIAL	OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)

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060502

EXAMINER	DATE CONSIDERED 9/3/04

*EXAMINER: INITIAL IF CITATION CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP 609, DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

FORM PTO-1449 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. SAEG108.001APC	APPLICATION NO. 10/070,938
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (USE SEVERAL SHEETS IF NECESSARY)	APPLICANT Morita, et al.	
	FILING DATE June 4, 2002	GROUP 1623

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE (IF APPROPRIATE)
	1	4,725,273	02/16/88	Kazuaki Kira	623	1	
	2						
	3						
	4						
	5						
	6						

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES	TRANSLATION NO
	7	JP 62-44260	02/26/87	Japan			Abstract	
	8							
	9							
	10							
	11							
	12							

EXAMINER INITIAL		OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)
	13	Shinoka, et al., "Creation of Viable Pulmonary Artery Autografts Through Tissue Engineering," The Journal of Thoracic and Cardiovascular Surgery, Vo. 115, No. 3, pp.536-545 March 1998
	14	
	15	
	16	
	17	

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091202

EXAMINER	DATE CONSIDERED 9/3/04

*EXAMINER: INITIAL IF CITATION CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP 609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Shinichiro Morita et al.
Appl. No.	:	10/070,938
Filed	:	June 4, 2002
For	:	MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND METHOD FOR REGENERATING CARDIOVASCULAR TISSUE
Examiner	:	NAFF, DAVID M.
Group Art Unit	:	4758

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

February 8, 2005
(Date)

Katsuhiro Arai, Reg. No. 43,315

AMENDMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Office Action mailed September 8, 2004, please reconsider the present application in light of the following amendments and comments.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

AMENDMENTS TO THE CLAIMS

Please amend the Claim Form and Claim as follows. Insertions are shown underlined while deletions are ~~struck through~~.

1 (original): A matrix for culturing cardiovascular cells to regenerate cardiovascular tissue comprising a sponge made of a bioabsorbable material and a reinforcement made of a bioabsorbable material.

2 (original): The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1, wherein the bioabsorbable material is at least one member selected from the group consisting of polyglycolic acid, polylactic acid (D form, L form, DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form) copolymer, glycolic acid-caprolactone copolymer, lactic acid (D form, L form, DL form)-caprolactone copolymer and poly(p-dioxanone).

3 (original): The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating an artery, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

4 (original): The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating a vein, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polyglycolic acid.

5 (original): The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1 for use in regenerating a cardiac valve or a pericardium, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

6 (original): The matrix for culturing cardiovascular cells to regenerate cardiovascular tissue according to Claim 1, wherein the sponge has a pore diameter of about 5 μm to about 100 μm.

7 (original): A method for regenerating cardiovascular tissue comprising seeding cells on the matrix of Claim 1 and culturing the cells.

8 (original): The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a blood vessel.

9 (original): The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a cardiac valve.

10 (original): The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a pericardium.

11 (original): The method for regenerating cardiovascular tissue according to Claim 7, wherein the cells to be seeded are a mixed cell culture of two or three different kinds selected from the group consisting of endothelial cells, smooth muscle cells and fibroblasts.

12 (new): A vascular prosthesis comprising the matrix for culturing cardiovascular cells of Claim 1 which is seeded with a cell culture and cultured in vitro.

13 (new): The vascular prosthesis according to Claim 12, wherein the cell culture is a mixed cell culture of two or three different kinds selected from the group consisting of endothelial cells, smooth muscle cells and fibroblasts.

14 (new): The vascular prosthesis according to Claim 12, wherein the matrix surface is completely covered with the cells.

REMARKS

Claims 12-14 have been added. Support for these claims can be found throughout the specification, for example, page 12. No new matter has been added. Applicant respectfully requests entry of the amendments and reconsideration of the application in view of the amendments and the following remarks.

Rejection Under 35 U.S.C. § 103

Claims 1-11 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Naughton et al in view of Vyakarnam et al taken with Hinsch et al and Japanese patent 3-23864. Applicant respectfully traverses the rejection.

The present invention is directed to, for example, a matrix for culturing cardiovascular cells to regenerate cardiovascular tissue comprising a sponge made of a bioabsorbable material and a reinforcement made of a bioabsorbable material, and to a method for regenerating cardiovascular tissue comprising seeding cells on said matrix and culturing the cells.

The present invention achieves in at least an embodiment the remarkable effect of obtaining a matrix which allows cells to sufficiently adhere thereto, provides an optimum scaffold for cell proliferation, maintains satisfactory blood flow resistance in vivo till autogeneous tissue is regenerated, and is ultimately decomposed and absorbed in vivo.

Cited References

Naughton discloses stromal cell-based three-dimensional living stromal tissues that can be used as corrective structures in the body, including tubular structures that can be used to replace or repair blood vessels (column 6, lines 55 to 58). Naughton discloses examples of biodegradable matrices, such as of polyglycolic acid, in column 9, lines 59 to 63 in the specification. A collagen sponge is disclosed in column 9, line 42 in the specification. Naughton discloses that, in addition to fibroblasts and other stromal cells, smooth muscle cells, endothelial cells and the like can also be used (column 4, lines 23 to 30).

Vyakarnam discloses porous bioabsorbable polymer foams that have a gradient in composition and/or microstructure (column 1, lines 8 to 13, and column 4, lines 10 to 25). These foams are useful for regeneration of tissues such as vascular grafts (column 3, lines 13 to 23). Vyakarnam also discloses, in column 9, lines 53 to 56, bioabsorbable polymers such as

copolymers of lactide. In column 4, line 67 to column 5, line 2, Vyakarnam discloses foams with pore size of 30 μm to 50 μm and 100 μm to 200 μm in porous gradient.

Hinsch discloses a porous (average pore size of 10-200 μm) implant suitable for growing blood vessels, the implant being made of a resorbable polymer such as polylactide, in which at least one textile reinforcement made of resorbable plastic is embedded (abstract; page 2, lines 1 to 4 and 43 to 45; and page 3, lines 8 to 13).

JP3-23864 discloses a filler to be transplanted in vivo, the filler comprises a collagen sponge in which a fibrous bioabsorbable polymer (poly-L-lactic acid) is embedded therein (Abstract). This filler accelerates the growth of fibroblasts, maintains its shape and strength for a duration enough long for medical treatment, and is absorbed in vivo after treatment.

No Motivation to Combine Naughton/Vyakarnam with Hinsch/JP3-23864

The Examiner has asserted that it would have been obvious to form the biodegradable polyglycolic acid copolymer tubular structure of Naughton with the biodegradable foam of Vyakarnam, and it would have been further obvious to reinforce the foam with fibers as suggested by Hinsch and JP3-23864. However, the present invention is not obvious from the cited references for the reasons described below.

Naughton teaches a biodegradable matrix having a tubular structure that can be used to replace or repair a blood vessel. However, it does not disclose a reinforcement for reinforcing the biodegradable matrix.

Vyakarnam teaches a porous bioabsorbable polymer foam having a tubular structure used for regenerating blood vessels, etc., and harvesting the cells by seeding them onto the foams (column 18, from line 58). However, it does not disclose a reinforcement made of a biodegradable polymer.

The implant of Hinsch is made of a resorbable polyester such as polylactide or the like, is used for growing blood vessels, is porous having pores of which the average pore diameter is 10-200 μm, and has a textile reinforcement formed of resorbable plastic embedded therein. Hinsch is different from the present invention in that it does not teach that cells are grown by being seeded on the implant.

The filler disclosed in JP3-23864 uses poly-L-lactic acid. However, JP3-23864 is different from the present invention in that this filler is embedded in vivo as is, i.e., without

seeding and growing cells, and that the filler is not used for regenerating blood vessel or the like tissues.

Considering the above, both Naughton and Vyakarnam employ "tissue engineering" in which tissues are regenerated by seeding, culturing, and growing autogenous cells on a matrix, and the regenerated tissues are transplanted in vivo. However, Hinsch and JP3-23864 relate to implants used by simply embedding the implants in. vivo without seeding, culturing, growing the cells onto a matrix. Therefore, they are fundamentally different from Naughton and Vyakarnam.

In other words, Naughton and Vyakarnam, which disclose a method for regenerating tissues by employing tissue engineering, are totally different from Hinsch and JP3-23864, which do not employ tissue engineering, in terms of the techniques used, role and function thereof, etc. Therefore, there is no motivation in Naughton and Vyakarnam to combine the teachings thereof with those of Hinsch and JP3-23864. In other words, it would not have been obvious to arrive at employing "a reinforcement made of a biodegradable material" with "the foam formed of a biodegradable material" of Naughton and Vyakarnam.

None of the References Disclose the Subject Matter of Claims 3-5

The invention claimed in Claims 3-5 is directed to a matrix comprising a sponge and reinforcement suitable for the matrix to regenerate an artery, vein, heart valve, or pericardia (claims 3, 4 and 5). The present invention defines a sponge and reinforcement suitable for the regeneration of specific cardiovascular tissues. None of the cited references include such disclosures or teachings.

Naughton and Vyakarnam disclose a matrix for blood vessels (artery and vein) and like tubular structures. However, they neither teach nor suggest that such a matrix can be used for regeneration of a "heart valve" or "pericardia" as recited in claims 5, 9 and 10 of the present invention. Therefore, these claims are unobvious.

Furthermore, as described in Example 1 in the present specification, artificial blood vessels with and without a reinforcement exhibited significant differences in working-effects when implanted in the inferior vena cava of a young dog. Specifically, the matrix not reinforced with poly-L-lactide fiber ruptured one week after substitution and the dog suddenly died. In contrast, no obliteration by rupture was found in the matrix with a reinforcement of the present invention and revealed regeneration of the autogenous blood vessel in agreement with the

transplantation site. Even a skilled artisan would not have expected such a remarkable effect from the above-mentioned cited references.

For the reasons described above, Applicant respectfully submits that the present invention is not obvious from the cited references, and requests that the pending claims be allowed.

New Claims

Claims 12-14 have been added. These claims recite "seeded with a cell culture and cultured in vitro." As explained above, none of the references teaches or even suggests a reinforce matrix seeded and cultured in vitro. For example, Hinsch et al and JP3-23864 simply teach embedding implants in vivo. Thus, the references could not lead to the invention recited in Claims 12-14. It is respectfully submitted that these claims are allowable.

CONCLUSION

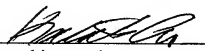
In light of the Applicant's amendments to the claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Feb. 8, 2005

By: 

Katsuhiro Arai
Registration No. 43,315
Attorney of Record
Customer No. 20,995
(949) 760-0404

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020805

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Applicant	:	Shinichiro Morita et al.
App. No	:	10/070,938
Filed	:	June 4, 2002
For	:	MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND METHOD FOR REGENERATING CARDIOVASCULAR TISSUE
Examiner	:	NAFF, DAVID M.
Art Unit	:	4758

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

February 8, 2005
(Date)

Katsuhiro Arai, Reg. No. 43,315

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Enclosed for filing in the above-identified application is a Supplemental Information Disclosure Statement by Applicant (PTO/SB/08 equivalent) listing 1 reference to be considered by the Examiner.

This Information Disclosure Statement is being filed before the mailing date of a final action and before the mailing of a Notice of Allowance. This Statement is accompanied by the fees set forth in 37 C.F.R. § 1.17(p). The Commissioner is hereby authorized to charge any additional fees which may be required or to credit any overpayment to Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Feb. 8, 2005

By:

Katsuhiro Arai
Registration No. 43,315
Attorney of Record
Customer No. 20,995
(949) 760-0404

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SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT		
	Application No.	10/070,938
	Filing Date	June 4, 2002
	First Named Inventor	Shinichiro Morita et al.
	Art Unit	4758
(Multiple sheets used when necessary)	Examiner	NAFF, DAVID M.
SHEET 1 OF 1	Attorney Docket No.	SAEG108.001APC

U.S. PATENT DOCUMENTS					
Examiner Initials	Cite No.	Document Number *Number - Kind Code (if known)* Example: 1,234,567 B1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear
		5,855,610	01-05-1999	Vacanti et al.	

FOREIGN PATENT DOCUMENTS						
Examiner Initials	Cite No.	Foreign Patent Document *Country Code-Number-Kind Code* Example: JP 1234567 A1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear	T[1]

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]

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020705

Examiner Signature	Date Considered

***Examiner:** Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

T[1] - Place a check mark in this area when an English language Translation is attached.

10/070,938 - 06/04/2002 - Morita - SAEG108.001APC - 4758
dea/kca/CPP

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/070,938	06/04/2002	Shinichiro Morita	SAEG108.001APC	4758

20995 7590 05/17/2005

KNOBBE MARTENS OLSON & BEAR LLP
2040 MAIN STREET
FOURTEENTH FLOOR
IRVINE, CA 92614

EXAMINER	
NAFF, DAVID M	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 05/17/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/070,938	MORITA ET AL.	
	Examiner	Art Unit	
	David M. Naff	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 February 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-14* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

An amendment of 2/11/05 in response to an office action of 9/8/04 added new claims 12-14.

Claims examined on the merits are 1-14, which are all claims in the application.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 103

Claims 1-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Naughton et al (5,863,531) in view of Vyakarnam et al (6,534,084 B1) taken with Hinsch et al (EP 0 274 898) and Japanese patent 3-23864 for reasons in the previous office action of 9/8/04, and for reasons herein.

The claims are drawn to a matrix for culturing cardiovascular cells to regenerate cardiovascular tissue comprising a sponge made of a bioabsorbable material and a reinforcement made of a bioabsorbable material. Also claimed is a method of culturing cardiovascular cells to regenerate cardiovascular tissue by seeding cells on the matrix and culturing the cells. The sponge has a pore diameter of about 5-100 μm. The matrix can be in the form of a vascular prosthesis seeded with a cell culture and cultured *in vitro*. The cell culture can be a mixture of two or more different kinds of cells. The matrix surface of the prosthesis can be completely covered with cells.

Naughton et al disclose producing tissue *in vitro* by seeding cells on a three-dimensional structure having interstitial spaces

which can be used to form tubular tissue structures (col 6, lines 55-60 and col 22, line 41) such as in the form of blood vessels (col 24, line 33), arteries (col 24, line 37) or veins (col 25, line 24). The three dimensional structure can be made of biodegradable material such as polyglycolic acid, polylactic acid or polyglycolic acid copolymer (col 9, lines 60-62). The three-dimensional structure can be made of a sponge (col 9, line 42).

Vyakarnam et al disclose foam structures that can be composed of copolymers of lactide such as a poly(L) lactide-co-E-caprolactone (col 6, line 45, col 9, lines 53-55 and col 12, lines 5-9), and which can be used to regenerate tissue such as tubular structures such as vascular grafts (col 3, lines 1 and 20-21, and col 9, lines 19-24). The pore size of the foam can be 30-50 Tm or 100-200 Tm (paragraph bridging cols 4 and 5). The foam can be reinforced with fibers (col 6, line 40).

Hinsch et al disclose a porous implant having a pore size of 10-200 Tm for the growth of blood vessels in the form of a foam made of a resorbable polymer such as a copolymer of glycolide and lactide (page 4, lines 1-8). The foam may contain textile reinforcing elements such as fibers or knitted fabrics (page 3, lines 8-13).

The Japanese patent discloses a reinforced collagen sponge for implanting in tissue. The sponge is reinforced with fibers made of poly-L-lactic acid.

It would have been obvious to form the biodegradable polyglycolic acid copolymer tubular structures of Naughton et al with the biodegradable foam of Vyakarnam et al that is a copolymer of glycolide and lactide since this foam can be used for producing a tubular structure and has advantageous properties. It would have been further obvious to reinforce the foam with fibers as suggested by Hinsch et al and the Japanese patent using fibers to reinforce a foam implant. It would have been obvious to use fibers formed of poly-L-lactic acid as taught by the Japanese patent so thqt both the foam and fibers are bioabsorbable. Using polyglycolic acid as in claim 4 to form the fibers would have been a matter of obvious choice. A foam as disclosed by the references is a sponge. When forming the tubular structures of Naughton et al, cardiovascular cells are used and the tissue produced is cardiovascular tissue. The conditions of dependent claims would have been obvious from the disclosures of the references. Structures other than tubular structures such as a cardiac valve or pericardium as in certain dependent claims would have been obvious from the many different structures disclosed by Naughton et al. A vascular prosthesis as in claim 12 would have been obvious from Naughton et al disclosing a tubular structure in the form of blood vessels, arteries or veins. Using two or more different cells as in claims 11 and 12 would have been obvious from Naughton et al using different types of cells together. Cells completely covering the matrix as in claim 14 is inherent with producing a tubular structure as taught by Naughton et al.

Response to Arguments

Applicant's arguments filed 2/11/05 have been fully considered but they are not persuasive.

Applicants urge that Naughton et al and Vyakarnam et al are drawn to tissue engineering, and are totally different from Hinsch et al and the Japanese patent which do not employ tissue engineering, and there is no motivation to combine the teachings of Naughton et al and Vyakarnam et al with those of Hinsch et al and the Japanese patent. However, since the foam of Vyakarnam et al, which is used for tissue engineering can be reinforced with fibers, it would have been apparent that reinforcement is important irrespective of whether tissue is produced on the foam or sponge before implanting. Even after tissue is engineered on the foam or sponge, it is implanted and reinforcement would have been expected to be important for the same type of reasons as when tissue is not produced on the foam or sponge before implanting. Moreover, the invention of claims 1-6 does not require tissue to be present, and the matrix can be implanting without seeding with cells and culturing to produce tissue.

As to claims 3-5 that applicants urge are not disclosed by the references, Vyakarnam et al disclose foam structures composed of poly(L) lactide-co-E-caprolactone, and the Japanese patent discloses a sponge reinforced with fibers made of poly-L-lactic acid. It would have been well within the skill of the art to use the foam of Vyakarnam et al in Naughton et al and reinforce the foam with the fibers of the Japanese patent. The use of polyglycolic acid for

reinforcement as in claim 4 would have also be obvious since this polymer would have been expected to provide the same function as polylactic acid when used to make fibers.

The results in Example 1 are unpersuasive, since the references suggest using fibers to reinforce tubular structures to be implanted, and it would have been obvious to reinforce the vessel before implanting. The references would not have taught reinforcement if reinforcement had not needed to provide additional strength.

As to claims 12-14, Naughton et al, as well as Vyakarnam et al, suggest seeding and culturing on a matrix to be implanted. The references are combined together and must be considered as whole in combination rather than each alone.

Conclusion

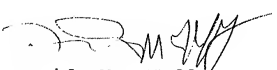
THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David M. Naff whose telephone number is 571-272-0920. The examiner can normally be reached on Monday-Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 751-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).



David M. Naff
Primary Examiner
Art Unit 1651

DMN
5/16/05

SAEG108.001APC

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Shinichiro Morita et al.
Appl. No.	:	10/070,938
Filed	:	June 4, 2002
For	:	MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND METHOD FOR REGENERATING CARDIOVASCULAR TISSUE
Examiner	:	David M. Naff
Group Art Unit	:	1651

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

September 19, 2005
(Date)

C. Philip Pointer, Reg. No. 43,006

AMENDMENT

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Final Office Action mailed May 17, 2005, please reconsider the present application in light of the following amendments and comments.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

AMENDMENTS TO THE CLAIMS

1-6. (Cancelled)

7. (Currently amended) A method for regenerating cardiovascular tissue comprising:

seeding cells on ~~the~~ a matrix ~~of Claim 1~~ comprising a sponge configured to regenerate cardiovascular tissue and made of a bioabsorbable material and a reinforcement made of a bioabsorbable material; ~~and~~

culturing the cells until the matrix surface is completely covered with the cells; and

embedding the matrix in vivo for regenerating cardiovascular tissue.

8. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a blood vessel.

9. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a cardiac valve.

10. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a pericardium.

11. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cells to be seeded are a mixed cell culture of two or three different kinds selected from the group consisting of endothelial cells, smooth muscle cells and fibroblasts.

12-14. (Cancelled)

15. (New) The method for regenerating cardiovascular tissue according to Claim 7, wherein the bioabsorbable material is at least one member selected from the group consisting of polyglycolic acid, polylactic acid (D form, L form, or DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form) copolymers, glycolic acid-caprolactone copolymers, lactic acid (D form, L form, DL form)-caprolactone copolymers and poly(p-dioxanone).

16. (New) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating an artery, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

17. (New) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating a vein, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polyglycolic acid.

18. (New) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating a cardiac valve or a pericardium, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

19. (New) The method for regenerating cardiovascular tissue according to Claim 7, wherein the sponge has a pore diameter of about 5 μm to about 100 μm.

REMARKS

In the Office Action, the Examiner maintained the rejection of the pending claims as obvious under 35 U.S.C. § 103 over Naughton, et al. (U.S. Patent No. 5,863,531) in view of Vyakarnam, et al. (U.S. Patent No. 6,534,084) taken with Hinsch, et al. (EP 0 274 898) and Morita (Japanese Patent No. 3-23864).

By this amendment, Claims 1-6 and 12-14 have been cancelled. Claim 7 has been amended to more clearly define the present invention. Support for amended Claim 7 may be found at page 3, line 21 – page 4, line 7; page 6, lines 4-8; and Example 1. Furthermore, Claims 15-19 have been added. Support for new Claims 15-19 may be found at page 4, line 8 – page 5, line 11; page 6, line 9 - page 7, line 12; and in originally-filed Claims 2-6. No new matter has been added thereby. Thus, Claims 7-11 and 15-19 are currently pending in the application. The Examiner's rejections are traversed below.

Rejection under 35 U.S.C. § 103

The Examiner has maintained his previous rejection of all of the pending claims under 35 U.S.C. § 103(a) as being unpatentable over Naughton, et al. (U.S. Patent No. 5,863,531) in view of Vyakarnam, et al. (U.S. Patent No. 6,534,084) taken with Hinsch, et al. (EP 0 274 898) and Morita (Japanese Patent No. 3-23864). The Examiner has repeated the grounds for rejection from the first Office Action and has indicated that Vyakarnam's disclosure of a foam reinforced with fibers shows a motivation to combine Naughton and Vyakarnam with Hinsch and Morita.

The Examiner's rejection rests on two central propositions. First, the Examiner indicates that "the invention of claims 1-6 does not require tissue to be present, and the matrix can be implanting without seeding with cells and culturing to produce tissue." Office Action at 5. In response, Applicant has amended the claims to require that cells be seeded onto the matrix and completely cover it prior to embedding the matrix in vivo. Second, the Examiner concludes that one of skill in the art would have understood from the cited references that reinforcement would be important even if tissue were grown on the matrix before it was implanted. Specifically, the Examiner states that

> since the foam of Vyakarnam et al, which is used for tissue engineering can be reinforced with fibers, it would have been apparent that reinforcement is

> important irrespective of whether tissue is produced on the foam or sponge before implanting. Even after tissue is engineered on the foam or sponge, it is implanted and reinforcement would have been expected to be important for the same type of reasons as when tissue is not produced on the foam or sponge before an implanting.

Id.

With respect to this second proposition, Applicant respectfully submits that, upon closer consideration, the references cited by the Examiner teach away from the claimed combination. In brief, Vyakarnam discloses the use of reinforcing fibers only in foam used to regenerate bone or cartilaginous tissue attached to bone, where greater stiffness is required. Applicant submits that the Examiner has failed to show compatibility between bone or cartilaginous tissue and cardiovascular tissue. Vyakarnam's disclosure of tissue scaffoldings used in vascular repair does not include the use of reinforcing fibers. Naughton specifically criticizes the use of artificial reinforcing materials in blood vessels, where elasticity is required. Applicant respectfully submits that the Examiner has failed to consider this teaching-away disclosure. Neither Hinsch nor Morita contemplate tissue engineering in which cells are grown in the foam before implantation in the body, and therefore they only suggest that an open-cell foam having no natural tissue therein, which might be expected to be weaker than the surrounding tissue, should be reinforced with fibers before implantation. Applicant submits that one of ordinary skill in the art would not culture cells on the foam or sponge before implanting unless absolutely required to do so, because it is extremely burdensome to culture cells in this manner. Furthermore, Morita makes clear that the presence of such fibers is only required until the tissue is regenerated. Applicant notes that the tissue-covered matrix is reinforced in the pending claims. Considering the disclosure of the references as a whole, Applicant submits that one of skill in the art reading these references would be discouraged from placing reinforcing fibers in a matrix used for generating cardiovascular tissue via in vitro tissue culture followed by in vivo transplantation. The cited references at least provide no motivation for doing so. The results obtained in Example 1 were therefore surprising and non-obvious.

The disclosure of each reference will be discussed in detail below.

Vyakarnam '084

As the Examiner indicates, Vyakarnam discloses foam structures that can be composed of copolymers of lactide and which can be used to regenerate tissue such as vascular grafts. Furthermore, as the Examiner notes, Vyakarnam teaches that the foam may be reinforced with fibers. Specifically, the discussion of fibers to which the Examiner refers is within a section of the Vyakarnam specification discussing cartilage. Here, however, the reinforcing fibers are restricted to a section of the cartilage that attaches to bone, in which sufficient stiffness is required:

> [A]t the bottom of this structure there is a need for larger pores (about 150 μm to about 300 μm) with higher stiffness to be structurally compatible with cancellous bone. The foam in this section could be reinforced with ceramic particles or fibers made up of calcium phosphates and the like.

Vyakarnam '084 at col. 6, lines 36-41 (emphasis added). Furthermore, Vyakarnam also discusses the use of fibers in tissue scaffoldings for bone repair, which would clearly also require significant stiffness. Vyakarnam '084 at col. 8, lines 5-12. But although Vyakarnam specifically describes possible tissue scaffoldings for use in skin and vascular repair, the reference does not disclose the use of artificial reinforcing fibers in such applications. By inference, Vyakarnam suggests to one of skill in the art that the use of reinforcing fibers is only preferable where higher stiffness is required, such as in those parts of cartilage which are connected to bone or in bone itself.

This understanding of Vyakarnam is reinforced by the discussion of Hinsch '898 therein. Vyakarnam discusses Hinsch in the "Background of the Invention" section, specifically noting that Hinsch teaches the reinforcement, with fibers or the like, of a porous open cell foam. Vyakarnam '084 at col. 1, lines 36-41. However, Vyakarnam then specifically criticizes Hinsch as deficient. Vyakarnam '084 at col. 1, lines 48-49.

Naughton '531

As the Examiner indicates, Naughton discloses foam structures that may be used in regenerating vasculature. However, Naughton does not teach the use of fibrous reinforcing materials such as those of the present application. In fact, Naughton specifically criticizes the use in the prior art of artificial materials to provide "the strength and elasticity required of blood vessels in vivo," and notes that the criticized prior art technique resulted in a construction that

"completely lacked elastin." Naughton '531 at col. 4, lines 2-6. The presence of elastin "gives arteries the ability to stretch with every contraction of the heart." Naughton '531 at col. 24, lines 48-50. Naughton therefore indicates that when natural components such as elastin are present in tubular biological replacement tissues grown on an unreinforced matrix, they may be used as replacement tissues in the body:

> The different biological structures described below have several features in common. They are all tubular structures primarily composed of layers of stromal tissue with an interior lining of epithelium (gastrointestinal and genitourinary) or endothelium (blood vessels). Their connective tissues also contain layers of smooth muscle with varying degrees of elastic fibers, both of which are especially prominent in arterial blood vessels. By including and sustaining these components in three-dimensional cultures according to the present invention, the tissues they compose can attain the special structural and functional properties they require for proper physiological functioning in vivo. They can then serve as replacements for damaged or diseased tubular tissues in a living body.

Naughton '531 at col. 22, lines 49-62. Naughton would accordingly suggest two things to one of skill in the art. One is that the replacement tissues grown on the disclosed unreinforced matrices or foams have sufficient strength to be implanted in the body as prosthetic cardiovascular tissue once the tissue culture is complete. The other is that it is not advisable to employ artificial reinforcing materials to supply the strength and elasticity necessary for blood vessels in vivo, as this may hinder the development of the cellular and extracellular components required for the regenerated blood vessels to properly function.

Hinsch '898

Hinsch discloses an implant made of a resorbable polyester such as polylactide or the like that has pores with an average pore diameter of 10-200 μm and has a textile reinforcement formed of resorbable plastic embedded therein. Hinsch does not teach or suggest that cells may be grown by being seeded on the implant prior to implantion. Rather, the Hinsch implant is designed to be implanted and then colonized by the surrounding tissue. In other words, Hinsch describes the problem solved by the invention as the need for an implant "which, despite the adequately open-cell structure to permit the growing-in of cells and blood vessels, ha[s] an adequate strength and particularly tensile strength." Hinsch '898 at p. 2, lines 51-53 (emphasis added). Hinsch does not suggest that cells can be seeded on the implant ex vivo. Accordingly,

one of skill in the art would understand from Hinsch that reinforcement of an open-cell foam implant may be necessary when cells are not seeded onto the implant prior to implantation in vivo.

Morita '864

An English-language translation of the Morita specification and claims is attached. As the Examiner notes, Morita discloses a filler material for use in vivo that uses poly-L-lactic acid. Like Hinsch, Morita does not disclose seeding and growing cells on the filler material prior to implantation. Neither does Morita disclose that the filler material is used for regenerating blood vessels or similar cardiovascular tissue structures.

Furthermore, Morita clearly indicates that reinforcement is not necessary when fully regenerated tissue is present. Specifically, the implant disclosed in Morita is said to "maintain[] its strength and shape over a long period of time until the regeneration of the tissue." Morita translation at page 5, lines 13-14 (Morita '864 at page 7, lines 5-7). One of skill in the art would accordingly understand from Morita that if tissue was generated ex vivo by tissue engineering techniques, artificial reinforcement of the implant would not be necessary.

Applicant submits that the combined teachings of these references would counsel one of skill in the art to avoid the use of artificial reinforcements in cardiovascular tissue engineered ex vivo. Vyakarnam's awareness of the possibility of using reinforcing fibers, and his choice to employ such fibers only where greater stiffness is required (as opposed to the elasticity required of blood vessels), together with Naughton's criticism of the use of artificial reinforcements in blood vessel prostheses, would indicate to one of skill in the art that use of reinforcing fibers was at best unnecessary, and probably unwise, as it might make the resulting prostheses too stiff or hinder the development of the natural components that provide elasticity. Both Hinsch and Morita indicate that the purpose of reinforcing fibers is simply to provide the mechanical strength which is lacking in an uncultured open-cell structure which is implanted into the body. *See* Hinsch '898 at page 2, lines 51-53 and page 3, lines 8-11; Morita translation at page 5, lines 13-14 (Morita '864 at page 7, lines 5-7). Indeed, Morita suggests that the fibers are only required when the tissue is not yet fully regenerated. Thus, these disclosures support the view that the reinforcing fibers are only necessary in a matrix for culturing cardiovascular tissue when the

matrix has not yet been cultured with cells, and that when such cells are present on the matrix, they would provide sufficient strength for the prosthetic tissue in vivo.

In view of these combined teachings, as described above, Applicant submits that one of skill in the art would have concluded that it was inadvisable, and at least unnecessary, to place artificial reinforcing fibers into a matrix seeded with cardiovascular cells to regenerate a blood vessel before implantation, as the presence of the fibers could make the prosthesis too stiff or impede the development of the cardiovascular tissue. Accordingly, the results obtained by the present invention, as shown in Example 1, are surprising and the invention is not obvious over the combination of cited references.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: September 19, 2005

By: C Philip P

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1932698
091405

MORITA ET AL., JP 3-23864

Specification

1. Title of the Invention

Filler material for living tissue

2. Claims

1. A filler material for living tissue, characterized in comprising a composite material of collagen sponge and a bioabsorbable polymer material.

2. A filler material for living tissue in accordance with Claim 1, characterized in that a fibrous bioabsorbable polymer material is mixed into or embedded in the collagen sponge.

3. A filler material for living tissue in accordance with Claim 1 or Claim 2, characterized in that the bioabsorbable polymer material is poly-L-lactic acid.

3. Detailed Description of the Invention

(Industrial Applicability)

The present invention relates to a filler material which may be employed in the surgical treatment of wounds and defects and the like or in orthopedic surgery.

(Background Art)

In the surgical treatment of wounds or defects or the like, and in orthopedic surgery, filler material is embedded in damaged areas in order to regenerate tissue and to prevent contracture.

It is required of such materials that they have little reactivity with tissue, that they promote the proliferation of fibroblasts, and that they maintain their strength and shape over a long period of time until the tissue is regenerated. Furthermore, it is a particularly required property that such materials maintain their shape in order to prevent contracture of the tissue

during actual use, and additionally, that they rapidly disappear from within the body and do not remain as a foreign object after the regeneration of the tissue.

Microporous collagen sponges have been proposed for such purposes; however, they do not have the above properties.

(Problem to be Solved by the Invention)

That is to say, collagen sponges in which, for example, glutaraldehyde is cross-linked do not maintain the requisite long-term shape and strength required for use in treatment, and within two to three months of three implantation in the body, they are completely broken down and absorbed by the body and disappear.

The present invention solves the defects present in the prior art; it provides a novel filler material having little reactivity with tissue and which promotes the propagation of fibroblasts, maintains its shape and strength over a long period of time, and furthermore is absorbed into the body after treatment.

(Means for Solving the Problem)

Moreover, the present invention is characterized in that it comprises a composite material consisting of collagen sponge and a biodegradable polymer material, fibrous poly-L-lactic acid is employed as the biodegradable polymer material, and this material is mixed into or embedded in the collagen sponge.

(Function)

By combining poly-L-lactic acid, which is slow to degrade within the body, with the collagen sponge, the present invention makes it possible to maintain the structural pores of the sponge over a long period of time, and furthermore, to promote the propagation of fibroblasts in

the interior of the material by means of the combination with fibrous poly-L-lactic acid, and also to maintain the strength and shape over the long period of time required for treatment.

Hereinbelow, the composition will be described.

(Embodiment)

0.3 g of 3-Denier poly-L-lactic acid fibers (molecular weight 80,000) were twined in a sliver, and placed in a vessel having length, width, and height dimensions of 6 x 2 x 2 cm, and this was agitated for a period of 60 minutes at 1,800 rpm with 50 g of a 0.3% hydrochloric acid solution of porcine atherocollagen. Next, this was freeze-dried for a period of 48 hours and sterilized in alcohol to produce the filler material of the present invention.

The filler material obtained in this manner had the appearance of a composite in which poly-L-lactic acid fibers were randomly embedded in a microporous sponge structure.

Furthermore, as shown in Table 1, in comparison with the prior art sponge composed only of collagen, the rupture strength, rupture ductility, and Young's modulus of the present invention are considerably higher, and it represents a dramatic improvement. Furthermore, the pore size is larger.

The comparative example in the Table is a sponge comprising only collagen that was prepared by a method identical to that described above using 50 g of a 0.2% hydrochloric acid solution of porcine atherocollagen, using glutaraldehyde as a crosslinking agent.

Table 1

	Strength	Ductility	Young's Modulus	Pore Size
Present invention	7.7	132	27.8	97
Comparative Example	1.3	40	8.0	63

These values were obtained by the JIS methods. Furthermore, the units are as given below.

Strength: rupture strength (x 10^5) (dyne/cm^2)

Ductility: rupture ductility (%)

Young's Modulus: (x 10^5) (dyne/cm^2)

Pore Size: (μm)

The filler material of the present invention obtained by the method described above was employed in animal testing using the following methods, and the histology, strength, and state of contracture thereof were assessed.

(Applied Example)

A 2 x 2 cm section of the back muscle of a 350 g Wistar rat was removed, and an approximately 2 cm section of the filler material of the present invention was implanted at this spot, and the progress thereof was observed.

(After One Month)

The infiltration of fibroblasts into the peripheral portions of the sponge was confirmed, but the cells had not infiltrated into the central portion thereof.

(After Three Months)

The cellular infiltration into the central section of the sponge was increased in comparison with after two months.

(After Six Months)

In portions of the central part of the sponge, the fibroblasts were arranged in a single direction.

Histologic studies revealed that fibroblasts had sufficiently penetrated the central part of the sponge three to four months after implantation, and the tissue was completely regenerated after six months.

The state of contracture was assessed using a method in which the volume was measured by means of plaster modeling. Using the comparative example above, only approximately 5-15% of the initial volume remained after two months, and after four months, the absorption into the body was complete, and the material had disappeared. In contrast, using the filler material of the present invention, 35-50% of the original volume was present, even after six months, and this represents a striking difference.

(Effects of the Invention)

As is clear from the effects obtained when the filler material of the present invention was applied, as described above, the material has the required properties for use and does not react with tissue, promotes the propagation of fibroblasts, maintains its strength and shape over a long period of time until the regeneration of the tissue, functions to prevent contracture of the tissue, and is broken down and absorbed into the body after the regeneration of tissue, so that the material has all the properties necessary for use, and may be effectively employed.

The proportions in which the collagen sponge and the bioabsorbable polymeric material are combined, as well as the size of the poly-L-lactic acid fibers and the like may be appropriately selected in accordance with the required properties.

As described above, the present invention provides a biodegradable filler material having a novel composition which was not conventionally available.

dca/KDA/CPP

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/070,938	06/04/2002	Shinichiro Morita	SAEG108.001APC	4758

20995 7590 10/12/2005

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EXAMINER	
NAFF, DAVID M	
ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 10/12/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Advisory Action Before the Filing of an Appeal Brief	Application No. 10/070,938	Applicant(s) MORITA ET AL.	
	Examiner David M. Naff	Art Unit 1651	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 22 September 2005 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☒ The period for reply expires 4 months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☐ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☐ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☐ They raise the issue of new matter (see NOTE below);
   (c) ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☒ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☒ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: *None*.
   Claim(s) objected to: *None*.
   Claim(s) rejected: *7-11 and 15-19*.
   Claim(s) withdrawn from consideration: *None*.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See Continuation Sheet.
12. ☐ Note the attached Information Disclosure Statement(s). (PTO/SB/08 or PTO-1449) Paper No(s). _____
13. ☐ Other: _____.

David M. Naff
Primary Examiner
Art Unit: 1651

Continuation of 11. does NOT place the application in condition for allowance because: of the following reasons. Applicants state that the claims have been amended to require seeding the matrix with cells and culturing until the matrix is completely covered with the cells before embedding the matrix in vivo. However, as set in the rejection, Naughton et al disclose seeding a matrix with cells and producing in vitro tissue such as a tubular tissue structure, and implanting the tissue structure. In producing such a tissue structure, the matrix will be completely covered with cells or otherwise the structure not be formed.

Applicants urge that Vyakarnam et al use reinforcing fibers only in foam when regenerating bone or cartilaginous tissue. However, cardiovascular tissue can contain cartilaginous tissue. Furthermore, Vyakarnam et al disclose producing a scaffold for vascular repair, and recognize (col 1, lines 38-45) that it is known to use foam reinforced with fibers for repair of blood vessels (Hinsch et al ('898). It would have been obvious when Hinsch et al is also considered to reinforce a scaffold used to produce tissue structures in the form of blood vessels or arteries as taught by Naughton et al. While Vyakarnam et al may consider Hinsch et al to be deficient in certain aspects, this is not related to the use of reinforcing fibers, but because the foam of Hinsch et al lacks the anisotropic features of natural tissues. In fact, Vyakarnam et al state that the Hinsch foams had an advantage of having regular pore sizes and shapes that could be controlled by the processing conditions, solvents selected, and the additives (col 1, lines 40-45).

Applicants urge that Naughton et al criticizes the use of artificial reinforcing material in blood vessels. However, the invention as broadly claimed in claims 7-11 and 19 does not require a reinforcing material that is artificial. The bioabsorbable material of claims 7-11 and 19 can be connective tissue proteins that serve as a support as disclosed by Naughton et al. As to the other claims that require the reinforcement to comprise polylactic acid or polyglycolic acid, such reinforcement would have been obvious when Hinsch et al and the Japanese patent are considered which teach reinforcement with fibers that can be made of polylactic acid. The use of synthetic polymer fibers for reinforcement is clearly obvious as an alternative to extracellular matrix protein such as elastin and collagen produced by cells that provide support in the matrix of Naughton et al. Naughton et al is not applied alone, but in combination with other references, and the references must be considered together as a whole rather than each alone. It would have been well within the ordinary skill of the art to select between two alternatives of providing support to a foam matrix. While Hinsch et al and the Japanese patent may not seed the foam with cells before implanting, seeding with cells is suggested by Naughton et al, as well as Vyakarnam et al (paragraph bridging cols 18 and 19), to provide a matrix containing tissue or cells in vitro prior to implanting. It is granted that the Japanese patent discloses that the scaffold (filler material) breaks down and is absorbed into the body after tissue is formed. However, the matrix of the present claims is bioabsorbable and will break down and be absorbed into the body when tissue is regenerated in vivo after implanting.

Contrary to applicants' assertion, there is no suggestion by the references that using artificial reinforcing fibers in a matrix seeded with cardiovascular cells will make the matrix too stiff or impede the development of the cardiovascular tissue. If too great a stiffness occurred, Vyakarnam et al, Hinsch et al and the Japanese patent would not have disclosed providing support for a sponge or foam to be implanted with synthetic polymer fibers. If the fibers impeded the development of tissue, the references would not have used the reinforcing fibers since the matrix of the references serves as a support for cells to form tissue even when the matrix is implanted without seeding with cells in vitro. After implanting, cells infiltrate the scaffold and form tissue in vivo. .

SAEG108.001APC asg
DEA/KOA/CPP

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/070,938	06/04/2002	Shinichiro Morita	SAEG108.001APC	4758

20995 7590 06/01/2006

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EXAMINER	
NAFF, DAVID M	
ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 06/01/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/070,938	MORITA ET AL	
	Examiner	Art Unit	
	David M. Naff	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *3/20/06*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *7-11 and 15-19* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *7-11 and 15-19* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on *04 June 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *2/11/05, 4/18/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

In view of the appeal brief filed on 3/20/06, PROSECUTION IS HEREBY REOPENED. New grounds of rejection are set forth below.

To avoid abandonment of the application, appellant must exercise one of the following two options:

(1) file a reply under 37 CFR 1.111 (if this Office action is non-final) or a reply under 37 CFR 1.113 (if this Office action is final); or,

(2) initiate a new appeal by filing a notice of appeal under 37 CFR 41.31 followed by an appeal brief under 37 CFR 41.37. The previously paid notice of appeal fee and appeal brief fee can be applied to the new appeal. If, however, the appeal fees set forth in 37 CFR 41.20 have been increased since they were previously paid, then appellant must pay the difference between the increased fees and the amount previously paid.

A Supervisory Patent Examiner (SPE) has approved of reopening prosecution by signing below.

Claims examined on the merits examined on the merits are 7-11 and 15-19, which are all claims in the application.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 103

Claims 7-9, 11, 15 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Vacanti et al (5,855,610) (newly applied).

The claims are drawn to a method for regenerating cardiovascular tissue by seeding cells on a matrix comprising sponge configured to regenerate cardiovascular tissue and made of bioabsorbable material and a reinforcement made of a bioabsorbable material, culturing the cells until the matrix surface is completely covered with cells, and embedding the matrix *in vivo* for generating cardiovascular tissue.

Vacanti et al disclose reconstruction and augmentation of flexible, strong connective tissue such as arteries and heart valves (col 1, lines 4-7). Objectives include producing tissue engineered constructs having improved mechanical strength and flexibility, making valves and vessels which can withstand repeated stress and strain, and improving yields of engineered tissues (col 2, lines 33-42). Structures are created by seeding a fibrous or porous polymeric matrix with cells (col 2, lines 65-67) to form tissues having structural elements such as heart valves and blood vessels (col 3, line 2-3). For a tissue to be constructed, successfully implanted and function, matrices must have sufficient surface area and exposure to nutrients such that cellular growth and differentiation can occur prior to the ingrowth of blood vessels following implantation (col 3, lines 26-29). The matrix acts as a scaffold providing a three-dimensional space for cell growth. The matrix functions as a template providing structural cues for tissue development (col 3, lines 10-15). The scaffold determines the limits of tissue growth and thereby determines the ultimate shape of a tissue engineered construct. The cells on the matrix proliferate only to the edges of the matrix (col 3, lines 20-

23). The matrix can be formed of polymers having a fibrous structure, which has sufficient interstitial spacing to allow for free diffusion of nutrients and gases to cells attached to the matrix surface. The spacing can be in a range of 100 to 300 microns, although closer spacings can be used if the matrix is implanted, blood vessels allowed to infiltrate the matrix, then the cells are seeded into the matrix (col 3, lines 42-49). The matrix can be sponge like (col 3, line 51), and can be a polyvinyl alcohol sponge (col 4, lines 25-27). The matrix can be formed of a biodegradable polymer such as poly(lactide) (PLA), poly(glycolic acid) (PGA) or poly(lactide-co-glycolide) (PLGA) (col 4, lines 8-11). Forms of lactic acid used to prepare PLA polymers can be L(+), D(-) or DL (col 4, lines 45-49). The overall matrix configuration is dependent on the tissue, which is to be constructed or augmented. The shape of the matrix can be obtained using struts that impart resistance to mechanical forces to yield the desired shape such as heart valve leaflets and tubes (col 3, lines 62-67). The struts can be biodegradable, and formed of the polymer material used to form the matrix to provide a matrix having sufficient strength to resist the necessary mechanical forces (col 5, lines 38-48). In Example 1 (beginning in col 7, line 60), a tissue engineered heart valve is produced. A PGA fiber based matrix is seeded with a mixed cell population containing myofibroblasts and endothelial cells and grown in culture until the myofibroblasts reached confluence. Then endothelial cells are seeded onto the surface of the fibroblast/mesh construct and grown into a single monolayer. The

tissue engineered heart valve resembled native valve tissue. The construct was implanted in sheep to determine if the construct had the required pliability and mechanical strength (col 8, lines 21-23). In Example 2 (beginning in col 8, line 45), a tissue engineered vascular structure is prepared. A PGA tubular construct is seeded with a smooth muscle cells and fibroblasts. After the fibroblasts and smooth muscle cells have grown to confluence, endothelial cells are seeded on the construct and the construct placed in culture (col 8, lines 50-56). Endothelially lined smooth muscle/fibroblast tubes were created (col 9, lines 5-7).

Vacanti et al disclose producing blood vessels, arteries and heart valves (cardiovascular tissue) using steps as claimed by seeding cells on a matrix made of bioabsorbable material configured to regenerate the tissue, culturing the cells on the matrix (Examples 1 and 2), and embedding the matrix *in vivo*, i.e. implanting the matrix containing tissue formed (col 2, lines 41-42, and col 8, line 21). It would have been obvious to reinforce the matrix with a bioabsorbable material since Vacanti et al disclose that the matrix must have sufficient mechanical strength, and that the mechanical strength can be obtained by providing the matrix with biodegradable struts to form a matrix having sufficient strength to resist mechanical forces. It would have been obvious to use the biodegradable polymer matrix of Vacanti et al in the form of a sponge since Vacanti et al disclose that the matrix can be sponge like (col 3, line 51) and the use of a polyvinyl alcohol sponge (col 4, lines 25-26). Growing cells to

confluence and forming a monolayer of endothelial cells on the matrix as in Example 1 of Vacanti et al (col 8, lines 12-15) will produce a matrix completely covered with cells as required in claim 7. Growing cells to confluence and culturing as in Example 2 of Vacanti et al will also result in the matrix completely covered with cells. Producing a blood vessel as in claim 8 and a cardiac valve as in claim 9 is disclosed by Vacanti et al. In Examples 1 and 2, Vacanti et al use a mixed cell culture (col 8, lines 8, and 49-50) as in claim 11. Vacanti et al disclose using materials that are bioabsorbable (col 4, lines 9-15 and 41-49) as in claim 15. A pore diameter of 100 μm is encompassed by the pore diameter range of about 5 μm to about 100 μm of claim 19, and 100 μm would have been obvious from Vacanti et al disclosing the matrix containing interstitial spacing of 100 to 300 microns for diffusion of nutrients and gases to cells (col 3, line 46).

Response to Arguments

The argument in the brief concerning tissue engineering and tissue requiring elasticity not being disclosed is unpersuasive with respect to Vacanti et al since Vacanti et al is clearly performing tissue engineering, and the tissue produced will have elasticity the same as tissue produced by the claimed process. Furthermore, the present claims do not require producing tissue having a certain amount of elasticity.

The argument in regard to the matrix of the claims being completely covered with cells is unpersuasive since Vacanti et al

disclose Examples 1 and 2 culturing under conditions that will completely cover the matrix. In particular, in Example 1, a monolayer of endothelial cells is formed as in the present specification (page 11, line 19). Additionally, growing a mixed culture to confluence as in Examples 1 and 2 (after 3 weeks in Example 2) will result in the matrix being completely covered with cells as in the present specification when culturing a mixed culture (page 12, lines 22-25).

The argument concerning the use of a bioabsorbable reinforcement is unpersuasive since Vacanti et al disclose the need for mechanical strength, and using struts made of a biodegradable synthetic polymer to provide the matrix with mechanical strength.

As to the argument concerning *ex vivo* tissue engineering, Vacanti et al disclose *ex vivo* tissue engineering since in Examples 1 and 2 culturing is *in vitro*. In any event, claim 7 does not require culturing to be *ex vivo*, and culturing can be *in vivo* to produce the matrix surface completely covered with cells.

The argument concerning combining references does apply to the present rejection since no references are combined with Vacanti et al.

The argument concerning unexpected results of the invention is unpersuasive since the tissue engineering process suggested by Vacanti et al will produce results the same as obtained from the claimed method.

Claim Rejections - 35 USC § 103

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Vacanti et al as applied to claims 7-9, 11, 15 and 19 above, and

further in view of Fofonoff et al (5,882,929) taken with Cox (6,719,789) or Love (5,509,930) (all newly applied).

The claim requires pericardium tissue as the cardiovascular tissue regenerated.

Fofonoff et al disclose (col 20, lines 53-67) seeding a matt with cells to repair, reconstruct or replace tissue, which can be pericardial tissue (colo 20, line 66).

Cox discloses (col 24, lines 39-60) producing a heart valve using pericardium tissue.

Love discloses (col 1, lines 40-42, and col 10, lines 8 and 22) using pericardium tissue to produce a prosthetic heart valve.

When producing a heart valve by tissue engineering as disclosed by Vacanti et al, it would have been obvious to produce pericardium tissue to form the heart valve as suggested by Fofonoff et al producing pericardium tissue using a cell seeded matt, and Cox or Love using pericardium tissue to produce heart valves. Pericardium tissue would have been expected to be an effective tissue for producing a heart valve since this is a known tissue for producing a prosthetic heart valve.

Claim Rejections - 35 USC § 103

Claims 16-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Vacanti et al as applied to claims 7-9, 11, 15 and 19 above, and further in view of Vyakarnam et al (6,534,084), and if necessary in further view of the Japanese patent.

The claims require producing an artery, a vein or a cardiac valve wherein the sponge comprises lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid or polyglycolic acid.

Vyakarnam et al disclose foam structures that can be composed of copolymers of lactide such as a poly(L) lactide-co-E-caprolactone (col 6, line 45, col 9, lines 53-55 and col 12, lines 5-9), and which can be used to regenerate tissue such as tubular structures such as vascular grafts (col 3, lines 1 and 20-21, and col 9, lines 19-24). The pore size of the foam can be 30-50 Tm or 100-200 Tm (paragraph bridging cols 4 and 5). The foam can be reinforced with fibers (col 6, line 40) made of calcium phosphate.

The Japanese patent discloses a reinforced collagen sponge for implanting in tissue. The sponge is reinforced with fibers made of poly-L-lactic acid.

When using biodegradable polymer struts for reinforcement and a biodegradable sponge as a matrix as suggested by Vacanti et al as set forth above, it would have been obvious to use polylactic acid or polyglycolic acid as the polymer forming the struts since Vacanti et al disclose these as biodegradable polymers that can be used to form the struts and sponge. It would have been further obvious to use lactic acid-caprolactone copolymer to form the sponge that can be the matrix of Vacanti et al since Vacanti et al disclose that the matrix can be formed of polylactic acid or poly(caprolactone) (col 4, lines 9-11), and Vyakarnam et al disclosing foam structures such as vascular

grafts formed of poly(L) lactide-co-E-caprolactone for use in tissue engineering. If needed, the Japanese patent would have suggested reinforcement of a sponge with polylactic acid fibers.

Claim Rejections - 35 USC § 103

Claims 7, 8 and 11 are are rejected under 35 U.S.C. 103(a) as being unpatentable over Naughton et al (5,863,531).

The claimed invention is described above.

Naughton et al disclose producing tissue *in vitro* by seeding cells on a three-dimensional framework having interstitial spaces, which can be shaped to assume the conformation of natural organs and their components (col 4, lines 63-64). The three-dimensional framework can be formed of biodegradable matrices such as collagen sponge (col 9, line 42), or polyglycolic acid or polylactic acid and copolymers thereof (col 9, lines 59-62). Tubular tissue structures can be formed (col 6, lines 55-60 and col 22, line 41) such as in the form of blood vessels (col 24, line 33), arteries (col 24, line 37) or veins (col 25, line 24). Implantation of a valve is also disclosed (col 19, line 49). Stromal cells such as fibroblasts or stromal cells in combination with other cells such as endothelial cells or smooth muscle cells (col 4, lines 23-28, and col 11, lines 9-25) are grown *in vitro* on the framework where the stromal cells and their naturally secreted extracellular matrix proteins and connective tissue proteins envelop the framework to form a three dimensional living stromal tissue (col 4, lines 30-44, col 7, lines 51-60, and col 11, line 64). Since the inner walls of arteries are rich in elastin, an arterial

stroma should contain a high concentration of smooth muscle cells which elaborate elastin (col 13, lines 28-31). The elastin provides strength and elasticity required of blood vessels *in vivo* (col 4, lines 2-9). Once the three dimensional tissue has reached the appropriate degree of growth, tissue-specific cells are inoculated on the stromal tissue, and can be grown on the stromal tissue *in vitro* to form a cultured counterpart of the native tissue prior to implantation *in vivo* (paragraph bridging cols 13 and 14, and col 14, lines 5-10). The cells chosen for inoculation depend on the tissue to be produced such as epithelium, endothelium and smooth muscle (col 14, lines 13-16). When producing arties, fibroblast cells and smooth muscle cells can be cultured to subconfluence on separate frameworks, the frameworks combined and the smooth muscle cells proliferated to produce elastin to simulate natural arterial walls. Thereafter, endothelial cells are seeded on top of an upper, elastin-rich layer, and incubated until they form a confluent layer (paragraph bridging cols 24 and 25, and col 25, lines 11-15).

When producing tubular tissue structures such are arteries, veins, blood vessels that are cardiovascular tissue as disclosed by Naughton et al, it would have been obvious to use collagen sponge as the framework in which cells are cultured to produce the tissue as suggested by Naughton et al (col 9, line 60). The collagen sponge is a sponge matrix as required by the present claims, and the method disclosed by Naughton et al when using collagen sponge is the same presently claimed. The extracellular matrix containing elastin

produced during culturing to form the stromal tissue will result in a bioabsorbable material that provides reinforcement as required by the present claims since Naughton et al disclose that elastin is a necessary component of blood vessels and provides strength (col 4, line 5) to the vessels, and is normal component of arteries (col 13, lines 28-31). After culturing tissue-specific cells on the stromal tissue contained by the collagen sponge, the sponge surface will be completely covered with cells since Naughton et al disclose that the tissue produced is a counterpart of native tissue prior to implantation (col 14, lines 7-10), and disclose culturing seeded endothelial cells on a elastin-rich layer to form a confluent layer (col 25, lines 13-15). A collagen sponge that is not completely covered with tissue formed by culturing the tissue-specific cells will not be a counterpart of native tissue. Naughton et al suggest a blood vessel (col 24, line 33) as required by claim 8, and a mixed cell culture (col 8, lines 16-17, and col 11, lines 9-15) as required by claim 11.

Response to Arguments

Applicants urge that Naughton et al disclose the framework being "substantially enveloped" by the cells, which is not completely covering a matrix with cells as claimed. However, Naughton et al also disclose that the stromal cells "envelop" the framework (col 11, line 64). Since "substantially" is not recited, Naughton et al intend that the framework can be completely covered with stromal cells as an alternative to being "substantially enveloped". In any event, the

framework being substantially enveloped as disclosed by Naughton et al is only in regard to growing stromal cells on the framework. After growing tissue specific cells on the stromal tissue resulting from growing stromal cells on the framework, the framework will be completely covered with cells since Naughton et al disclose obtaining a cultured counterpart of native tissue (col 14, lines 6-8), obtaining a confluent layer of endothelial cells (col 25, lines 8-10), and an elastin-rich stromal culture lined with endothelium (col 25, lines 18-20). The present claims do not exclude culturing stromal cells on the matrix followed by culturing tissue-specific cells as disclosed by Naughton et al. The present specification discloses (page 11, lines 7-19) culturing fibroblasts (stromal cells) on the matrix, and then culturing endothelial cells to form a monolayer.

Applicants' argument concerning elasticity is unpersuasive since the claims do not require an elasticity different than obtained by Naughton et al. The claims encompass cells that produce a high content of elastin as is apparent by claim 11 reciting "smooth muscle cells" as cells that can be used in a mixed cell culture.

Applicants argue that Naughton et al teach away from using artificial materials to provide strength and elasticity (col 4, lines 2-6). However, Naughton et al do not teach that artificial materials should be excluded, but that the presence of elastin provides better results than when elastin is not present since elastin is present in the natural counterpart. In any event, the present claims do not require the reinforcement to be with an artificial material. The

bioabsorbable material used for reinforcement as claimed can be an extracellular matrix containing elastin as disclosed by Naughton et al. The presently claimed invention will not produce results unexpectedly different than obtained by Naughton et al. Vyakarnam et al, Hinsch et al and the Japanese patent are not applied in the present rejection, and arguments concerning these reference are moot with respect to the present rejection.

Claim Rejections - 35 USC § 103

Claim 9, 15 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Naughton et al as applied to claims 7, 8 and 11 above, and further in view of Vacanti et al.

The claimed invention, Naughton et al and Vacanti et al are described above.

It would have been obvious to use the procedure of Naughton et al to produce a heart valve as in claim 9 in view of Vacanti et al producing vascular structures or heart valves by a procedure similar to that of Naughton et al. Using bioabsorbable materials of claim 15 to produce a sponge instead of from collagen and to produce a reinforcement in addition to extracellular matrix containing elastin in Naughton et al would have been suggested by Vacanti et al using such materials to produce a biodegradable sponge-like matrix and a biodegradable reinforcement (struts) in a procedure similar to that of Naughton et al. Since Vacanti et al can use smooth muscle cells that produce elastin (col 8, line 50), it would have been apparent that reinforcement can be desirable even when elastin is present. A pore

size in the range of claim 19 would been obvious from Vacanti et al disclosing spacings of 100 to 300 microns (col 3, line 46).

Claim Rejections - 35 USC § 103

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over the references as applied to claims 9 and 15 above, and further in view of Fofonoff et al taken with Cox or Love.

The claimed invention and references are described above.

When producing a heart valve by the procedure of Naughton et al as suggested by Vacanti et al as set forth above, it would have been obvious to produce pericardium tissue to form the heart valve as suggested by Fofonoff et al producing pericardium tissue using a cell seeded matt, and Cox or Love using pericardium tissue to produce a heart valve. Pericardium tissue would have been expected to be an effective tissue for producing a heart valve since this is a known tissue for producing a prosthetic heart valve.

Claim Rejections - 35 USC § 103

Claims 16-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over the references as applied to claims 9, 15 and 19 above, and further in view of Vyakarnam et al, and if necessary in further view of the Japanese patant.

The invention and references described above.

When modifying Naughton et al as suggested by Vacanti et al as set forth above, it would have been obvious to use polylactic acid or polyglycolic acid as the polymer forming the struts since Vacanti et al disclose these as biodegradable polymers that can be used to form

the struts and sponge. It would have been further obvious to use lactic acid-caprolactone copolymer to form the sponge that can be the framework of Naughton et al as suggested by Vacanti et al since Vacanti et al disclose that the matrix can be formed of polylactic acid or poly(caprolactone) (col 4, lines 9-11), and Vyakarnam et al disclose foam structures such as vascular grafts formed of poly(L) lactide-co-E-caprolactone for use in tissue engineering. If needed, the Japanese patent would have suggested reinforcement of a sponge with polylactic acid fibers.

Response to Arguments

As set forth above, Naughton et al completely cover the framework with cells. Even if Vyakarnam et al does not completely cover the foam structure, this does not prevent completely covering the structure as suggested by Naughton et al.

Applicants urge that there is not motivation to combine bioabsorgable reinforcement with tubular foam structures. However, there is clear motivation. Vyakarnam et al, and if needed the Japanese patent, clearly suggest reinforcement of a sponge to be implanted. If reinforcement was not advantageous, the references would not have suggested using reinforcement. It is clear from Vacanti et al that reinforcement with artificial means can be used event when using smooth muscle cells that produce elastin. The fact that the Japanese patent may not use *ex vivo* tissue engineering does not make the rejection untenable since Naughton et al suggest *in vitro* growing of cells in a matrix to produce tissue. The references are

combined together, and must be considered together as a whole rather than each alone.

Bell et al (4,546,500) is made of record to further show reinforcement of engineered vessels.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David M. Naff whose telephone number is 571-272-0920. The examiner can normally be reached on Monday-Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mike Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

David M. Naff
Primary Examiner
Art Unit 1651

DMN

5/24/06

Michael G. Wityshyn
Supervisory Patent Examiner
Technology Center 1600

PTO/SB/08 Equivalent

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT BY APPLICANT		
	Application No.	10/070,938
	Filing Date	June 4, 2002
	First Named Inventor	Shinichiro Morita et al.
	Art Unit	4758
(Multiple sheets used when necessary)	Examiner	NAFF, DAVID M.
SHEET 1 OF 1	Attorney Docket No.	SAEG108.001APC

U.S. PATENT DOCUMENTS

Examiner Initials	Cite No.	Document Number *Number - Kind Code (if known)* Example: 1,234,567 B1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear
		5,855,610	01-05-1999	Vacanti et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials	Cite No.	Foreign Patent Document *Country Code-Number-Kind Code* Example: JP 1234567 A1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear	T[1]

NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]

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020705

Examiner Signature	D. M. Naff	Date Considered	5/15/06

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

T[1] - Place a check mark in this area when an English language Translation is attached.

PTO/SB/08 Equivalent

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Multiple sheets used when necessary)

SHEET 1 OF 1

Application No.	10/070,938
Filing Date	June 4, 2002
First Named Inventor	Shinichiro Morita
Art Unit	1651
Examiner	David M. Naff
Attorney Docket No.	SAEG108.001APC

U.S. PATENT DOCUMENTS

Examiner Initials	Cite No.	Document Number *Number - Kind Code (if known)* Example: 1,234,567 B1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear
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FOREIGN PATENT DOCUMENTS

Examiner Initials	Cite No.	Foreign Patent Document *Country Code-Number-Kind Code* Example: JP 1234567 A1	Publication Date MM-DD-YYYY	Name of Patentee or Applicant	Pages, Columns, Lines Where Relevant Passages or Relevant Figures Appear	T[1]
	9					
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	11					
	12					
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NON PATENT LITERATURE DOCUMENTS

Examiner Initials	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[1]
	14	EUROPEAN SEARCH REPORT DATED JANUARY 26, 2006 FOR APPLICATION NO. EP 00 95 7031	
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Examiner Signature	Date Considered 5/15/06

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

T[1] - Place a check mark in this area when an English language Translation is attached.

Notice of References Cited	Application/Control No. 10/070,938	Applicant(s)/Patent Under Reexamination MORITA ET AL	
	Examiner David M. Naff	Art Unit 1651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,719,789 B2	04-2004	Cox, James L.	623/2.13
*	B	US-5,509,930	04-1996	Love, Jack W.	623/2.1
*	C	US-5,882,929	03-1999	Fofonoff et al.	435/395
*	D	US-4,546,500	10-1985	Bell, Eugene	435/1.1
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Shinichiro Morita et al.
Appl. No.	:	10/070,938
Filed	:	June 4, 2002
For	:	MATRIX FOR REGENERATING CARDIOVASCULAR TISSUE AND METHOD FOR REGENERATING CARDIOVASCULAR TISSUE
Examiner	:	David M. Naff
Group Art Unit	:	1651

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

December 1, 2006
(Date)

C. Philip Porter, Reg. No. 43,006

AMENDMENT

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the non-final Office Action mailed June 1, 2006, please reconsider the present application in light of the following amendments and comments.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

AMENDMENTS TO THE CLAIMS

1-6. (Cancelled)

7. (Currently amended) A method for regenerating cardiovascular tissue comprising:

seeding cells on a matrix comprising a sponge configured to regenerate cardiovascular tissue and made of a bioabsorbable material and a reinforcement made of a bioabsorbable material, the reinforcement being integrated with the sponge and located inside or on the exterior surface of the matrix;

culturing the cells until the matrix surface is completely covered with the cells; and

embedding the matrix in vivo for regenerating cardiovascular tissue.

8. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a blood vessel.

9. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a cardiac valve.

10. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cardiovascular tissue to be regenerated is a pericardium.

11. (Original) The method for regenerating cardiovascular tissue according to Claim 7, wherein the cells to be seeded are a mixed cell culture of two or three different kinds selected from the group consisting of endothelial cells, smooth muscle cells and fibroblasts.

12-14. (Cancelled)

15. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7, wherein the bioabsorbable material is at least one member selected from the group consisting of polyglycolic acid, polylactic acid (D form, L form, or DL form), polycaprolactone, glycolic acid-lactic acid (D form, L form, DL form) copolymers, glycolic acid-caprolactone copolymers, lactic acid (D form, L form, DL form)-caprolactone copolymers and poly(p-dioxanone).

16. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating an artery, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

17. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating a vein, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polyglycolic acid.

18. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7 for use in regenerating a cardiac valve or a pericardium, wherein the sponge comprises a lactic acid-caprolactone copolymer and the reinforcement comprises polylactic acid.

19. (Previously presented) The method for regenerating cardiovascular tissue according to Claim 7, wherein the sponge has a pore diameter of about 5 μm to about 100 μm.

REMARKS

In the Office Action, the Examiner reopened the prosecution of the application. In response, Applicant elects to file this reply under 37 C.F.R. § 1.111.

The Examiner has rejected Claims 7-11 and 15-19 as obvious under 35 U.S.C. § 103(a) in view of one or more prior art references.

By this amendment, Claim 7 has been amended to more clearly define the present invention. The support for amended Claim 7 may be found at page 8, lines 16-22 of the specification. No new matter has been added thereby. Accordingly, Claims 7-11 and 15-19 are currently pending in the application. The Examiner's rejections are traversed below.

Claim Rejections - 35 U.S.C. § 103 (Vacanti)

The Examiner has rejected Claims 7-9, 11, 15, and 19 under 35 U.S.C. § 103(a) as being unpatentable over Vacanti, et al., U.S. Patent No. 5,855,610.

As amended, Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." Vacanti neither discloses nor suggests such a reinforcement.

As noted by the Examiner, see Office Action at 4, the only disclosure in Vacanti that corresponds to the claimed reinforcements are the "struts," which appear to be discussed only at col. 3, lns. 62-67 and col. 5, lns. 34-48. The struts are described as imparting "resistance to mechanical forces", thereby "yielding the desired shape." Vacanti '610 at col. 3, lns. 65-66. The exemplary applications of such struts are "heart valve 'leaflets' and tubes." *Id.* at col. 3, lns. 66-67. The single paragraph in which the struts are disclosed in detail is reproduced below:

> In some embodiments it may be desirable to create additional structure using devices provided for support, referred to herein as "struts." These can be biodegradable or non-degradable polymers which are inserted to form a more defined shape than is obtained using the cell-matrices. An analogy can be made to a corset, with the struts acting as "stays" to push the surrounding tissue and skin up and away from the implanted cells. In a preferred embodiment, the struts are implanted prior to or at the time of implantation of the cell-matrix structure. The struts are formed of a polymeric material of the same type as can be used to form the matrix, as listed above, having sufficient strength to resist the necessary mechanical forces.

The structural relationship between the struts and the cell-matrix structure is not specifically disclosed in this paragraph. Nevertheless, the struts are said to function in the manner of corset stays, exerting force on the tissue and skin surrounding an implanted graft to push it "up and

away from the implanted cells" (emphasis added). The struts also may be implanted prior to the implantation of the cell-matrix structure. These disclosures strongly suggest that rather than being "integrated with the sponge", as required by amended Claim 7, the struts are entirely separate from the cell-matrix structure. Thus, the only suggestion in this reference is to use a matrix unlike that presently claimed.

Moreover, there is also no disclosure or suggestion in the cited prior art reference that the struts should be located inside the matrix or on the exterior surface thereof, as required by amended Claim 7. If the struts were located within the cell-matrix structure, for example, they would be unable to perform the disclosed function of "push[ing] the surrounding tissue and skin up and away from the implanted cells." Rather, at least some of the cells would be forced against the surrounding tissue by action of the internal struts. Similarly, struts that were integral with and on the exterior surface of the cell-matrix structure would leave the implanted cells at the surface of the matrix in contact with the surrounding tissue or skin, which is inconsistent with the disclosed function of pushing the tissue or skin "away from" the matrix.

With respect to the examples cited by the Examiner at pages 4-5 of the Office Action, Applicant notes that neither example discloses the use of the "struts" that the Examiner equates with the claimed reinforcements. In Example 2, cardiovascular tissue (a blood vessel, as in pending Claim 8) was engineered, and the extracellular matrices of the resulting tissue were examined to determine if the engineered tissue had "the physical characteristics of native vascular tissues." Vacanti '610 at col. 9, lns. 7-11. The results showed that vascular tissue had been "successfully formed" without the use of reinforcements. *Id.* at lns. 13-14. Moreover, the engineered heart valve (as in pending Claim 9) of Example 1 was actually implanted into a sheep "to determine if the constructs had the required pliability and mechanical strength for use in patient." Vacanti '610 at col. 8, lns. 21-23. The results of the implantation are not given in the Vacanti reference. From this inclusion of this working example in the patent specification, however, one of skill in the art would assume that the unreinforced engineered heart valve performed adequately. In other words, Vacanti '610 discloses the engineering of unreinforced cardiovascular tissues that were found to have the physical characteristics of native tissue, and were actually tested in vivo and found to have the "required pliability and mechanical strength" for use in human beings. This testing would indicate to one of skill in the art that engineered cardiovascular tissue needed no reinforcement at all, let alone the specific claimed reinforcement

that is integral with the sponge of the matrix and inside or on the exterior surface thereof, as required by amended Claim 7. Vacanti '610 thus indicates (as does Naughton '531, as discussed below) that the replacement tissues grown on the disclosed unreinforced matrix have sufficient strength to be implanted in the body as prosthetic cardiovascular tissue once the tissue culture is complete. One of skill in the art would simply not be motivated to modify the cell matrices of Vacanti '610 in the manner set forth in amended Claim 7. As such, the Vacanti '610 reference fails to support a *prima facie* showing of obviousness.

Furthermore, even had a *prima facie showing* of obusness been set forth, the presently claimed invention provides unexpected advantages that would effectively rebut such a showing. The integration of the claimed reinforcement with the sponge and locating it either inside or on the exterior surface of the matrix, as required by amended Claim 7, provides the unexpected advantages that a smooth sponge surface comes into contact with the blood flow, which is advantageous in that it reduces the likelihood that thromboses will be formed, enhances cell adhesion, promotes the smooth supply of nutrition to the cells, and enhances the formation of the tunica intima. These effects of the present invention could not have been expected from Vacanti '610 by a person skilled in the art. Accordingly, Vacanti '610 neither discloses nor suggests to one of skill in the art the use of a reinforcement which is integrated with the sponge and located inside or on the exterior surface of the matrix, as required by amended Claim 7. Accordingly, Vacanti '610 does not make obvious the invention of amended Claim 7.

Furthermore, Claims 8-11 and 15-19 depend from Claim 7, and thus incorporate all of the limitations thereof, as well as further limitations. For these reasons, Claims 7-9, 11, 15, and 19 are not obvious over Vacanti '610.

Claim Rejections - 35 U.S.C. § 103 (Vacanti/Fofonoff/Cox or Love)

The Examiner has rejected Claim 10 over the combination of Vacanti '610 with Fofonoff et al., U.S. Patent No. 5,882,929, taken with Cox, U.S. Patent No. 6,719,789, or Love, U.S. Patent No. 5,509,930. As described above, Vacanti '610 does not disclose or suggest a reinforcement that is integrated with the sponge and located inside or on the exterior surface of the matrix, as required by amended Claim 7. Fofonoff '929, Cox '789, and Love '930 do not disclose or suggest the use of reinforcements in the production of cardiovascular tissue, let alone the use of the specific claimed reinforcements. Accordingly, they cannot supply the disclosure

lacking in Vacanti '610. For that reason, Claim 10 is not obvious over this combination of references.

Claim Rejections - 35 U.S.C. § 103 (Vacanti/Vyakarnam/Morita)

The Examiner has rejected Claims 16-18 as obvious over Vacanti '610 and further in view of Vyakarnam, U.S. Patent No. 6,534,084, and Morita, Japanese Patent No. 3-23864. As described above, amended Claim 7 requires the use of a reinforcement made of a bioabsorbable material which is integrated with the sponge and located inside or on the exterior surface of the matrix. As described above, Vacanti '610 does not disclose or suggest these limitations. Neither do Vyakarnam '084 or Morita '864 disclose or suggest such a reinforcement for use in cardiovascular tissue.

As the Examiner indicates, Vyakarnam '084 discloses foam structures that can be composed of copolymers of lactide and which can be used to regenerate tissue, such as vascular grafts. Furthermore, as the Examiner notes, Vyakarnam teaches that the foam may be reinforced with fibers made of calcium phosphate. The discussion of fibers to which the Examiner refers is within a section of the Vyakarnam specification discussing cartilage. Here, however, the reinforcing fibers are restricted to a section of the cartilage that attaches to bone, in which sufficient stiffness is required:

> [A]t the bottom of this structure there is a need for larger pores (about 150 μm to about 300 μm) with higher stiffness to be structurally compatible with cancellous bone. The foam in this section could be reinforced with ceramic particles or fibers made up of calcium phosphates and the like.

Vyakarnam '084 at col. 6, lines 36-41 (emphasis added). Furthermore, Vyakarnam also discusses the use of fibers in tissue scaffoldings for bone repair, which would clearly also require significant stiffness. Vyakarnam '084 at col. 8, lines 5-12. But although Vyakarnam specifically describes possible tissue scaffoldings for use in vascular repair, the reference does not disclose the use of reinforcements in such applications. Vyakarnam accordingly suggests to one of skill in the art that the use of reinforcing fibers is only preferable where higher stiffness is required, such as in those parts of cartilage which are connected to bone or in bone itself, and that reinforcement should not be used for cardiovascular tissue. Much less does Vyakarnam suggest to one of skill in the art the use of the specific claimed reinforcement of amended Claim 7, which is integrated with the sponge and located inside or on the exterior surface of the matrix.

Furthermore, Morita '864 does not supply what is lacking in Vacanti '610 and Vyakarnam '084. An English-language translation of Morita '864 was previously submitted along with the amendment and response of September 19, 2005. Morita discloses a filler material for use in vivo that uses poly-L-lactic acid. Morita does not disclose seeding and growing cells on the filler material prior to implantation. Neither does Morita disclose that the filler material is used for regenerating blood vessels or similar cardiovascular tissue structures.

Morita clearly indicates that reinforcement is not necessary when fully regenerated tissue is present. Specifically, the implant disclosed in Morita is said to "maintain[] its strength and shape over a long period of time until the regeneration of the tissue." Morita translation at page 5, lines 13-14 (Morita '864 at page 7, lines 5-7). One of skill in the art would accordingly understand from Morita that if tissue was generated ex vivo by tissue engineering techniques, artificial reinforcement of the implant would not be necessary.

Moreover, Morita does not disclose or suggest that the reinforcement should be specifically located inside or on the exterior surface of the matrix, as required by the amended claims. Accordingly, the combination of references cited by the Examiner fail to support a *prima facie* showing of obviousness with respect to Claims 16-18, which are accordingly not obvious over this combination of references.

Claim Rejections - 35 U.S.C. § 103 (Naughton)

The Examiner has rejected Claims 7, 8, and 11 as obvious over Naughton et al., U.S. Patent No. 5,863,531. As amended, Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." Naughton '531 neither discloses nor suggests such a reinforcement.

As the Examiner indicates, Naughton discloses foam structures that may be used in regenerating vasculature. However, Naughton does not teach the use of fibrous reinforcing materials, much less the specific reinforcement of amended Claim 7, which is integrated with the sponge and located inside or on the exterior surface of the matrix. Rather, Naughton indicates that tubular biological replacement tissues grown on an unreinforced matrix may be used as cardiovascular replacement tissues in vivo. The examples of tubular biological tissue engineering provided all use unreinforced mesh as the starting point. *See* Naughton '531 at col. 23. Furthermore, Naughton discloses that arterial structures grown in this way produce elastin, so as to "simulate . . . natural arterial walls." Naughton '531 at col. 25, lns. 10-11. Naughton

would accordingly suggest to one of skill in the art that the replacement tissues grown on unreinforced matrices or foams have sufficient strength to be implanted in the body as prosthetic cardiovascular tissue once the tissue culture is complete. Naughton thus does not disclose reinforcement of a matrix used to regenerate cardiovascular tissue.

Much less does Naughton disclose or suggest the specific reinforcement required by amended Claim 7, which is integrated with the sponge and located inside or on the exterior surface of the matrix. The Examiner states that "the extracellular matrix containing elastin produced during culturing to form the stromal tissue will result in a bioabsorbable material that provides reinforcement." Office Action at pages 11-12. However, elastin produced by pre-seeding the matrix with stromal cells as suggested by the Examiner will not constitute a reinforcement "integrated with the sponge," as required by amended Claim 7. Naughton '531 neither discloses nor suggests a reinforcement that is integrated with the sponge and located inside or on the exterior surface of the matrix. Therefore, Claim 7 is not obvious over Naughton '531.

Claims 8 and 11 depend from Claim 7, and thus incorporate all of the limitations thereof, as well as further limitations. For these reasons, Claims 8 and 11 are not obvious over Naughton '531.

Claim Rejections - 35 U.S.C. § 103 (Naughton/Vacanti)

The Examiner has rejected Claims 9, 15 and 19 as obvious over the combination of Naughton '531 and Vacanti '610. As amended, Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." As described above, neither Naughton '531 nor Vacanti '610 discloses or suggests the use of a matrix comprising such a reinforcement. Accordingly, Claims 9, 15, and 19 are not obvious over the combination of Naughton '531 and Vacanti '610.

Claim Rejections - 35 U.S.C. § 103 (Naughton/Vacanti/Fofonoff/Cox or Love)

The Examiner has rejected Claim 10 as obvious over the combination of Naughton '531 and Vacanti '610, and in view of Fofonoff '929, Cox '789, and Love '930. As amended, Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." As described above, none of Naughton '531, Vacanti '610, Fofonoff '929, Cox '789, or Love '930 discloses or suggests the use of a matrix comprising such a reinforcement. Accordingly, Claim 10 is not obvious over this combination of references.

Claim Rejections - 35 U.S.C. § 103 (Naughton/Vacanti/Vyakarnam/Morita)

The Examiner has rejected Claims 16-18 as obvious over the combination of Naughton '531, Vacanti '610, Vyakarnam '084, and Morita '864. As amended, Claim 7 limits the claimed reinforcement to one that is "integrated with the sponge and located inside or on the exterior surface of the matrix." As described above, none of Naughton '531, Vacanti '610, Vyakarnam '084, or Morita '864 disclose or suggest the use of a matrix comprising such a reinforcement. Accordingly, Claims 16-18 are not obvious over this combination of references.

Bell et al., 4,546,500

The Examiner has made Bell '500 of record "to further show reinforcement of engineered vessels." Office Action at 17. Applicant notes that Bell does not disclose a reinforcement made of a bioabsorbable material, as required by amended Claim 7. Rather, Bell incorporates a plastic mesh sleeve of Dacron, Teflon, or nylon. Bell '500 at col. 4, lns. 22-40.

CONCLUSION

In summary, integrating a reinforcement with the sponge and locating it either inside or on the exterior surface of the matrix, as required by amended Claim 7, provides sufficient strength to resist the pressure caused by blood flow in vivo, as shown by the in vivo animal study results disclosed on page 13 of the specification. In addition, the claimed invention provides the further unexpected advantages that a smooth sponge surface comes into contact with the blood flow, which is advantageous in that it reduces the likelihood that thromboses will be formed, enhances cell adhesion, promotes the smooth supply of nutrition to the cells, and enhances the formation of the tunica intima. These effects of the present invention could not have been expected from any of the prior art references, alone or in combination, by a person skilled in the art. Accordingly, the cited art neither discloses nor suggests to one of skill in the art the use of a reinforcement which is integrated with the sponge and located inside or on the exterior surface of the matrix, as required by amended Claim 7. Furthermore Claims 8-11 and 15-19 depend from Claim 7, and thus incorporate all of the limitations thereof, as well as further limitations. For these reasons, Claims 7-11 and 15-19 are not obvious over the cited art.

In view of the foregoing, the application is believed to be fully in condition for allowance, and allowance of the application is therefore respectfully requested. Should any remaining

impediments to allowance be identified by the Examiner, the Examiner is respectfully invited to contact the undersigned attorney at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 1 DECEMBER 2006

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/070,938	06/04/2002	Shinichiro Morita	SAEG108.001APC	4758

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2040 MAIN STREET
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EXAMINER	
NAFF, DAVID M	
ART UNIT	PAPER NUMBER
1657	

SHORTENED STATUTORY PERIOD OF RESPONSE	NOTIFICATION DATE	DELIVERY MODE
3 MONTHS	03/12/2007	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Notice of this Office communication was sent electronically on the above-indicated "Notification Date" and has a shortened statutory period for reply of 3 MONTHS from 03/12/2007.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

jcartee@kmob.com
eOAPilot@kmob.com

Office Action Summary	Application No.	Applicant(s)	
	10/070,938	MORITA ET AL.	
	Examiner	Art Unit	
	David M. Naff	1657	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 December 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *7-11 and 15-19* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *7-11 and 15-19* is/are rejected..

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

An amendment of 12/6/06, in response to an office action of 6/1/06, amended claim 7.

Claims examined on the merits are 7-11 and 15-19, which are all claims in the application.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 7-11 and 15-19 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In line 6 of claim 7, "reinforcement being integrated with the sponge" is uncertain as to meaning and scope. The difference in structure when the reinforcement is integrated with the sponge as compared to when the reinforcement is not integrated with the sponge is uncertain. While the specification (page 8, lines 16-17) discloses that reinforcement is preferably integrated with the sponge, the specification fails to define the difference between being integrated and not integrated with the sponge.

Claim Rejections - 35 USC § 103

Claims 7-9, 11 and 15-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Vacanti et al (5,855,610) in view of Vyakarnam et al (6,534,084) and Japanese patent (JP 3-23864) (Morita et al).

The claims are drawn to a method for regenerating cardiovascular tissue by seeding cells on a matrix comprising sponge configured to regenerate cardiovascular tissue and made of bioabsorbable material and a reinforcement made of a bioabsorbable material integrated with the sponge and located inside or on the exterior surface of the matrix, culturing the cells until the matrix surface is completely covered with cells, and embedding the matrix *in vivo* for generating cardiovascular tissue.

Vacanti et al disclose reconstruction and augmentation of flexible, strong connective tissue such as arteries and heart valves (col 1, lines 4-7). Objectives include producing tissue engineered constructs having improved mechanical strength and flexibility, making valves and vessels which can withstand repeated stress and strain, and improving yields of engineered tissues (col 2, lines 33-42). Structures are created by seeding a fibrous or porous polymeric matrix with cells (col 2, lines 65-67) to form tissues having structural elements such as heart valves and blood vessels (col 3, line 2-3). For a tissue to be constructed, successfully implanted and function, matrices must have sufficient surface area and exposure to nutrients such that cellular growth and differentiation can occur prior to the ingrowth of blood vessels following implantation (col 3, lines 26-29).

The matrix acts as a scaffold providing a three-dimensional space for cell growth. The matrix functions as a template providing structural cues for tissue development (col 3, lines 10-15). The scaffold determines the limits of tissue growth and thereby determines the ultimate shape of a tissue engineered construct. The cells on the matrix proliferate only to the edges of the matrix (col 3, lines 20-23). The matrix can be formed of polymers having a fibrous structure, which has sufficient interstitial spacing to allow for free diffusion of nutrients and gases to cells attached to the matrix surface. The spacing can be in a range of 100 to 300 microns, although closer spacings can be used if the matrix is implanted, blood vessels allowed to infiltrate the matrix, then the cells are seeded into the matrix (col 3, lines 42-49). The matrix can be sponge like (col 3, line 51), and can be a polyvinyl alcohol sponge (col 4, lines 25-27). The matrix can be formed of a biodegradable polymer such as poly(lactide) (PLA), poly(glycolic acid) (PGA) or poly(lactide-co-glycolide) (PLGA) (col 4, lines 8-11). Forms of lactic acid used to prepare PLA polymers can be L(+), D(-) or DL (col 4, lines 45-49). The overall matrix configuration is dependent on the tissue, which is to be constructed or augmented. The shape of the matrix can be obtained using struts that impart resistance to mechanical forces to yield the desired shape such as heart valve leaflets and tubes (col 3, lines 62-67, and col 5, lines 35-48). The struts can be biodegradable, and formed of the polymer material used to form the matrix to provide a matrix having sufficient strength to resist the necessary mechanical

forces. In Example 1 (beginning in col 7, line 60), a tissue engineered heart valve is produced. A PGA fiber based matrix is seeded with a mixed cell population containing myofibroblasts and endothelial cells and grown in culture until the myofibroblasts reached confluence. Then endothelial cells are seeded onto the surface of the fibroblast/mesh construct and grown into a single monolayer. The tissue engineered heart valve resembled native valve tissue. The construct was implanted in sheep to determine if the construct had the required pliability and mechanical strength (col 8, lines 21-23). In Example 2 (beginning in col 8, line 45), a tissue engineered vascular structure is prepared. A PGA tubular construct is seeded with a smooth muscle cells and fibroblasts. After the fibroblasts and smooth muscle cells have grown to confluence, endothelial cells are seeded on the construct and the construct placed in culture (col 8, lines 50-56). Endothelially lined smooth muscle/fibroblast tubes were created (col 9, lines 5-7). Vacanti et al disclose producing blood vessels, arteries and heart valves (cardiovascular tissue) using steps as claimed by seeding cells on a matrix made of bioabsorbable material configured to regenerate the tissue, culturing the cells on the matrix (Examples 1 and 2), and embedding the matrix *in vivo*, i.e. implanting the matrix containing tissue formed (col 2, lines 41-42, and col 8, line 21).

Vyakarnam et al disclose foam structures that can be composed of copolymers of lactide such as a poly(L) lactide-co-E-caprolactone (col 6, line 45, col 9, lines 53-55 and col 12, lines 5-9), and which can

be used to regenerate tissue such as tubular structures such as vascular grafts (col 3, lines 1 and 20-21, and col 9, lines 19-24). The pore size of the foam can be 30-50 Tm or 100-200 Tm (paragraph bridging cols 4 and 5). The foam can be reinforced with fibers (col 6, line 40) made of calcium phosphate.

The Japanese patent discloses a reinforced collagen sponge for implanting in tissue. The sponge is reinforced with fibers made of poly-L-lactic acid. See the translation (page 3, 4th paragraph). The sponge is used as a filler material embedded in a wound of defect to regenerate tissue (page 1 and paragraph bridging pages 1 and 2 of translation).

It would have been obvious to use a sponge as the matrix of Vacanti et al as suggested by Vyakarnam et al using foam structures that can be composed of copolymers of lactide such as a poly(L) lactide-co-E-caprolactone to regenerate tissue such as tubular structures such as vascular grafts, and the Japanese patent treating wounds and defects by using collagen sponge as a filler material embedded in damaged areas to regenerate tissue, and as suggested by Vacanti et al disclosing that the matrix used can be sponge-like (col 3, line 51) and using polyvinyl alcohol sponge as the matrix (col 4, lines 25-26). It would have been obvious to provide reinforcement of the sponge with a bioabsorbable material since Vacanti et al disclose that the matrix must have sufficient mechanical strength, and that the mechanical strength can be obtained by providing the matrix with

biodegradable struts to form a matrix having sufficient strength to resist mechanical forces. It would have been obvious to provide the reinforcement with fibers in the sponge as suggested by Vyakarnam et al using fibers in foam structures for reinforcement (col 6, line 40) and the Japanese patent using fibers inside a collagen sponge to maintain shape and strength (page 2 of translation). The fibers would have been expected to provide the shaping and strengthing function of the struts of Vacanti et al, and the fibers can be considered to be struts. Vacanti et al disclose that the struts can be implanted at the same time as the matrix (col 5, line 44), and having the fibers integrated with the matrix would have been obvious. Growing cells to confluence and forming a monolayer of endothelial cells on the matrix as in Example 1 of Vacanti et al (col 8, lines 12-15) will produce a matrix completely covered with cells as required in claim 7. Growing cells to confluence and culturing as in Example 2 of Vacanti et al will also result in the matrix completely covered with cells. Producing a blood vessel, as in claim 8 and a cardiac valve as in claim 9, is disclosed by Vacanti et al. In Examples 1 and 2, Vacanti et al use a mixed cell culture (col 8, lines 8, and 49-50) as in claim 11. Vacanti et al disclose using materials that are bioabsorbable (col 4, lines 9-15 and 41-49) as in claim 15. A pore diameter of 100 μm is encompassed by the pore diameter range of about 5 μm to about 100 μm of claim 19, and 100 μm would have been obvious from Vacanti et al disclosing the matrix containing interstitial spacing of 100 to 300 microns for diffusion of nutrients and gases to cells (col 3, line

46). As to claims 16-18, it would have been obvious to use polylactic acid or polyglycolic acid as the polymer forming the struts or fibers since Vacanti et al disclose these as biodegradable polymers that can be used to form the struts and sponge. It would have been further obvious to use lactic acid-caprolactone copolymer to form the sponge that can be the matrix of Vacanti et al since Vacanti et al disclose that the matrix can be formed of polylactic acid or poly(caprolactone) (col 4, lines 9-11), and Vyakarnam et al disclosing foam structures such as vascular grafts formed of poly(L) lactide-co-E-caprolactone for use in tissue engineering. The Japanese patent would have suggested reinforcement of a sponge with polylactic acid fibers.

Response to Arguments

Arguments in the amendment urge that the struts of Vacanti et al are entirely separate from the cell-matrix structure. However, Vacanti et al disclose that the struts and matrix can be implanted at the same time, and Vyakarnam et al and the Japanese patent disclose fibers in a foam or sponge for reinforcement. Having struts or fibers integrated with the matrix as to be part of the matrix of Vacanti et al seeded with cells would have been obvious to provide reinforcement. The disclosure of the struts pushing surrounding tissue by Vacanti et al is not disclosed by Vacanti et al to be a critical function of the struts. Vacanti et al disclose that the struts provide strength and shape to the matrix, and it would have been obvious to provide the struts or fibers as part of the matrix for these functions without pushing tissue.

The arguments urge that the examples of Vacanti et al do not disclose using struts, and the matrix of Vacanti et al after cell growth has sufficient strength. However, the struts disclosed by Vacanti et al are intended for use where the matrix does not have sufficient strength. The examples of Vacanti et al use a fiber-based matrix such as a PGA mesh (col 8, line 11), which has sufficient strength without the struts. Fibers in one part of the mesh apparently acted as a reinforcement for fibers in another part of the mesh. There is no description in the present specification to establish that the matrix in Example 1 not reinforced was a fiber mesh as disclosed by the examples of Vacanti et al. In Example 1 in the present specification, when the plain-weave cloth is omitted as reinforcement, there are no fibers present as contained by the fiber mesh of Vacanti et al. Obviously, the non-reinforced matrix used for comparison in Example 1 will not have the strength of the fiber mesh used in the examples of Vacanti et al. When using a non-fiber mesh matrix such as a sponge having less strength than the fiber mesh, it would have been obvious to provide reinforcement as suggested by Vacanti et al, Vyakarnam et al and the Japanese patent.

The arguments in the amendment urge that Vyakarnam et al suggest using reinforcing fibers only where higher stiffness is required. However, the matrix not reinforced in Example 1 in the present specification is less stiff than the sponge containing the plain-weave cloth. The stiffness disclosed by Vyakarnam et al provides the matrix

with greater strength, and would have been expected to make the matrix more resistant to rupture as occurs in Example 1.

The arguments urge that the Japanese patent does not seed the filler with cells, and that reinforcement is not needed after fully regenerated tissue is present. However, the sponge used to produce the regenerated tissue is reinforced with fibers to maintain strength and shape during production of the tissue, and after the tissue has been regenerated the sponge has been absorbed into the body. The present claims do not require the matrix to contain regenerated tissue, but instead require the matrix to be covered with cells before embedding in vivo. Since the matrix is bioabsorbable, the matrix will be absorbed into the body as disclosed by the Japanese patent when tissue is regenerated.

Claim Rejections - 35 USC § 103

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Vacanti et al as applied to claims 7-9, 11 and 15-19 above, and further in view of Fofonoff et al (5,882,929) taken with Cox (6,719,789) or Love (5,509,930).

The claim requires pericardium tissue as the cardiovascular tissue regenerated.

Fofonoff et al disclose (col 20, lines 53-67) seeding a matt with cells to repair, reconstruct or replace tissue, which can be pericardial tissue (colo 20, line 66).

Cox discloses (col 24, lines 39-60) producing a heart valve using pericardium tissue.

Love discloses (col 1, lines 40-42, and col 10, lines 8 and 22) using pericardium tissue to produce a prosthetic heart valve.

When producing a heart valve by tissue engineering as disclosed by Vacanti et al, it would have been obvious to produce pericardium tissue to form the heart valve as suggested by Fofonoff et al producing pericardium tissue using a cell seeded matt, and Cox or Love using pericardium tissue to produce heart valves. Pericardium tissue would have been expected to be an effective tissue for producing a heart valve since this is a known tissue for producing a prosthetic heart valve.

Response to Arguments

Arguments in the amendment urge that Vacanti et al does not disclose or suggest reinforcement that is integrated with the sponge, and Fofonoff et al taken with Cox or Love do not suggest such reinforcement. However, for reasons set forth above, Vacanti et al, Vyakarnam et al and the Japanese patent suggest reinforcement integrated with the matrix. Fofonoff et al and Cox or Love are not relied on to suggest reinforcement.

Claim Rejections - 35 USC § 103

Claims 7, 8 and 11 are are rejected under 35 U.S.C. 103(a) as being unpatentable over Naughton et al (5,863,531).

The claimed invention is described above.

Naughton et al disclose producing tissue *in vitro* by seeding cells on a three-dimensional framework having interstitial spaces, which can be shaped to assume the conformation of natural organs and

their components (col 4, lines 63-64). The three-dimensional framework can be formed of biodegradable matrices such as collagen sponge (col 9, line 42), or polyglycolic acid or polylactic acid and copolymers thereof (col 9, lines 59-62). Tubular tissue structures can be formed (col 6, lines 55-60 and col 22, line 41) such as in the form of blood vessels (col 24, line 33), arteries (col 24, line 37) or veins (col 25, line 24). Implantation of a valve is also disclosed (col 19, line 49). Stromal cells such as fibroblasts or stromal cells in combination with other cells such as endothelial cells or smooth muscle cells (col 4, lines 23-28, and col 11, lines 9-25) are grown *in vitro* on the framework where the stromal cells and their naturally secreted extracellular matrix proteins and connective tissue proteins envelop the framework to form a three dimensional living stromal tissue (col 4, lines 30-44, col 7, lines 51-60, and col 11, line 64). Since the inner walls of arteries are rich in elastin, an arterial stroma should contain a high concentration of smooth muscle cells which elaborate elastin (col 13, lines 28-31). The elastin provides strength and elasticity required of blood vessels *in vivo* (col 4, lines 2-9). Once the three dimensional tissue has reached the appropriate degree of growth, tissue-specific cells are inoculated on the stromal tissue, and can be grown on the stromal tissue *in vitro* to form a cultured counterpart of the native tissue prior to implantation *in vivo* (paragraph bridging cols 13 and 14, and col 14, lines 5-10). The cells chosen for inoculation depend on the tissue to be produced such as epithelium, endothelium and smooth muscle (col 14, lines 13-

16).. When producing arties, fibroblast cells and smooth muscle cells can be cultured to subconfluence on separate frameworks, the frameworks combined and the smooth muscle cells proliferated to produce elastin to simulate natural arterial walls. Thereafter, endothelial cells are seeded on top of an upper, elastin-rich layer, and incubated until they form a confluent layer (paragraph bridging cols 24 and 25, and col 25, lines 11-15).

When producing tubular tissue structures such are arteries, veins, blood vessels that are cardiovascular tissue as disclosed by Naughton et al, it would have been obvious to use collagen sponge as the framework in which cells are cultured to produce the tissue as suggested by Naughton et al (col 9, line 60). The collagen sponge is a sponge matrix as required by the present claims, and the method disclosed by Naughton et al when using collagen sponge is the same presently claimed. The extracellular matrix containing elastin produced during culturing stromal cells will result in the extracellular matrix being integrated with the matrix and functioning for reinforcement prior to seeding the matrix with tissue specific cells. Naughton et al disclose that elastin is a necessary component of blood vessels and provides strength (col 4, line 5) to the vessels, and is normal component of arteries (col 13, lines 28-31). After culturing tissue-specific cells on the stromal tissue contained by the collagen sponge, the sponge surface will be completely covered with cells since Naughton et al disclose that the tissue produced is a counterpart of native tissue prior to implantation (col 14, lines 7-

10), and disclose culturing seeded endothelial cells on a elastin-rich layer to form a confluent layer (col 25, lines 13-15). A collagen sponge that is not completely covered with tissue formed by culturing the tissue-specific cells will not be a counterpart of native tissue. Naughton et al suggest a blood vessel (col 24, line 33) as required by claim 8, and a mixed cell culture (col 8, lines 16-17, and col 11, lines 9-15) as required by claim 11.

Response to Arguments

Arguments in the amendment urge that Naughton et al do not teach a fibrous reinforcing material integrated with the sponge. However, claim 7 does requirement a "fibrous reinforcing material". The reinforcing material can be any material capable of reinforcing. The extracellular matrix containing elastin produced during culturing stromal cells as disclosed by Naughton et al will be inside and/or on the surface of the sponge, and will be integrated with the matrix before the matrix is seeded with tissue specific cells. The present claims do not exclude the reinforced matrix before seeding with cells being a matrix previously used to culture stromal cells as disclosed by Naughton et al. Additionally, the claims do not specify how the reinforcement is integrated with the matrix. The claims encompass the reinforcement being integrated with the matrix by producing the reinforcement in the matrix during a step of cell culture before seeding followed by culturing tissue specific cells on the matrix as disclosed by Naughton el al. The extracellular matrix of Naughton et

al is integrated with the matrix before the tissue specific cells are added to the matrix.

Claim Rejections - 35 USC § 103

Claim 9, 15 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Naughton et al as applied to claims 7, 8 and 11 above, and further in view of Vacanti et al.

The claimed invention, Naughton et al and Vacanti et al are described above.

It would have been obvious to use the procedure of Naughton et al to produce a heart valve as in claim 9 in view of Vacanti et al producing vascular structures or heart valves by a procedure similar to that of Naughton et al. Using bioabsorbable materials of claim 15 to produce a sponge instead of from collagen and to produce a reinforcement in addition to extracellular matrix containing elastin in Naughton et al would have been suggested by Vacanti et al using such materials to produce a biodegradable sponge-like matrix and a biodegradable reinforcement (struts) in a procedure similar to that of Naughton et al. Since Vacanti et al can use smooth muscle cells that produce elastin (col 8, line 50) and use struts for reinforcement, it would have been apparent that reinforcement such as struts can be desirable even when elastin is present. A pore size in the range of claim 19 would been obvious from Vacanti et al disclosing spacings of 100 to 300 microns (col 3, line 46).

Response to Arguments

The arguments urge that Naughton et al and Vacanti et al do not disclose reinforcement integrated with the sponge, and located inside or on the exterior surface of the matrix. However, for reasons set forth above, Naughton et al suggest reinforcement integrated with the sponge.

Claim Rejections - 35 USC § 103

Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over the references as applied to claims 9 and 15 above, and further in view of Fofonoff et al taken with Cox or Love.

The claimed invention and references are described above.

When producing a heart valve by the procedure of Naughton et al as suggested by Vacanti et al as set forth above, it would have been obvious to produce pericardium tissue to form the heart valve as suggested by Fofonoff et al producing pericardium tissue using a cell seeded matt, and Cox or Love using pericardium tissue to produce a heart valve. Pericardium tissue would have been expected to be an effective tissue for producing a heart valve since this is a known tissue for producing a prosthetic heart valve.

Response to Arguments

The type of response to arguments set forth above in regard to claims 9, 15 and 19 also applies to arguments traversing this rejection.

Claim Rejections - 35 USC § 103

Claims 16-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over the references as applied to claims 9, 15 and 19 above, and further in view of Vyakarnam et al, and if necessary in further view of the Japanese patant.

The invention and references described above.

When modifying Naughton et al as suggested by Vacanti et al as set forth above, it would have been obvious to use polylactic acid or polyglycolic acid as the polymer forming the struts since Vacanti et al disclose these as biodegradable polymers that can be used to form the struts and sponge. It would have been further obvious to use lactic acid-caprolactone copolymer to form the sponge that can be the framework of Naughton et al as suggested by Vacanti et al since Vacanti et al disclose that the matrix can be formed of polylactic acid or poly(caprolactone) (col 4, lines 9-11), and Vyakarnam et al disclose foam structures such as vascular grafts formed of poly(L) lactide-co-E-caprolactone for use in tissue engineering. If needed, the Japanese patent would have suggested reinforcement of a sponge with polylactic acid fibers.

Response to Arguments

The type of response to arguments set forth above in regard to claims 9, 15 and 19 also applies to arguments traversing this rejection.

Bell et al (4,546,500) is made of record to further show reinforcement of engineered vessels.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David M. Naff whose telephone number is 571-272-0920. The examiner can normally be reached on Monday-Friday 9:30-6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jon Weber can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

David M. Naff
Primary Examiner
Art Unit 1657

DMN
3/5/07

US005855610A

United States Patent [19]

Vacanti et al.

[11] Patent Number: 5,855,610

[45] Date of Patent: Jan. 5, 1999

[54] **ENGINEERING OF STRONG, PLIABLE TISSUES**

[75] Inventors: **Joseph P. Vacanti**, Winchester; **Christopher K. Breuer**, Brighton; **Beverly E. Chaignaud**; **Toshiraru Shin'oka**, both of Brookline, all of Mass.

[73] Assignee: **Children's Medical Center Corporation**, Boston, Mass.

[21] Appl. No.: **445,280**

[22] Filed: **May 19, 1995**

[51] **Int. Cl.**[6] **A61F 2/02**

[52] **U.S. Cl.** **623/11**

[58] **Field of Search** 623/1, 2, 11, 12, 623/66; 424/425, 426

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[57] **ABSTRACT**

It has been discovered that improved yields of engineered tissue following implantation, and engineered tissue having enhanced mechanical strength and flexibility or pliability, can be obtained by implantation, preferably subcutaneously, of a fibrous polymeric matrix for a period of time sufficient to obtain ingrowth of fibrous tissue and/or blood vessels, which is the removed for subsequent implantation at the site where the implant is desired. The matrix is optionally seeded prior to the first implantation, after ingrowth of the fibrous tissue, or at the time of reimplantation. The time required for fibrous ingrowth typically ranges from days to weeks. The method is particularly useful in making valves and tubular structures, especially heart valves and blood vessels.

9 Claims, No Drawings

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ENGINEERING OF STRONG, PLIABLE TISSUES

BACKGROUND OF THE INVENTION

This invention is generally in the field of reconstruction and augmentation of flexible, strong connective tissue such as arteries and heart valves.

Tissue engineering is a multidisciplinary science that utilizes basic principles from the life sciences and engineering sciences to create cellular constructs for transplantation. The first attempts to culture cells on a matrix for use as artificial skin, which requires formation of a thin three dimensional structure, were described by Yannas and Bell (See, for example, U.S. Pat. Nos. 4,060,081, 4,485,097 and 4,458,678). They used collagen type structures which were seeded with cells, then placed over the denuded area. A problem with the use of the collagen matrices was that the rate of degradation is not well controlled. Another problem was that cells implanted into the interior of thick pieces of the collagen matrix failed to survive.

U.S. Pat. No. 4,520,821 to Schmidt describes the use of synthetic polymeric meshes to form linings to repair defects in the urinary tract. Epithelial cells were implanted onto the synthetic matrices, which formed a new tubular lining as the matrix degraded. The matrix served a two fold purpose - to retain liquid while the cells replicated, and to hold and guide the cells as they replicated.

In European Patent Application No. 88900726.6 "Chimeric Neomorphogenesis of Organs by Controlled Cellular Implantation Using Artificial Matrices" by Children's Hospital Center Corporation and Massachusetts Institute of Technology, a method of culturing dissociated cells on biocompatible, biodegradable matrices for subsequent implantation into the body was described. This method was designed to overcome a major problem with previous attempts to culture cells to form three dimensional structures having a diameter of greater than that of skin. Vacanti and Langer recognized that there was a need to have two elements in any matrix used to form organs: adequate structure and surface area to implant a large volume of cells into the body to replace lost function and a matrix formed in a way that allowed adequate diffusion of gases and nutrients throughout the matrix as the cells attached and grew to maintain viability in the absence of vascularization. Once implanted and vascularized, the porosity required for diffusion of the nutrients and gases was no longer critical.

To overcome some of the limitations inherent in the design of the porous structures which support cell growth throughout the matrix solely by diffusion, WO 93/08850 "Prevascularized Polymeric Implants for Organ Transplantation" by Massachusetts Institute of Technology and Children's Medical Center Corporation disclosed implantation of relatively rigid, non-compressible porous matrices which are allowed to become vascularized, then seeded with cells. It was difficult to control the extent of ingrowth of fibrous tissue, however, and to obtain uniform distribution of cells throughout the matrix when they were subsequently injected into the matrix.

Many tissues have now been engineered using these methods, including connective tissue such as bone and cartilage, as well as soft tissue such as hepatocytes, intestine, endothelium, and specific structures, such as ureters. There remains a need to improve the characteristic mechanical and physical properties of the resulting tissues, which in some cases does not possess the requisite strength and pliability to perform its necessary function in vivo. Examples of particular structures include heart valves and blood vessels.

Despite major advances in its treatment over the past thirty-five years, valvular heart disease is still a major cause of morbidity and mortality in the United States. Each year 10,000 Americans die as a direct result of this problem. Valve replacement is the state-of-the art therapy for end-stage valve disease. Heart valve replacement with either nonliving xenografts or mechanical protheses is an effective therapy for valvular heart disease. However, both types of heart valve replacements have limitations, including finite durability, foreign body reaction or rejection and the inability of the non-living structures to grow, repair and remodel, as well as the necessity of life-long anticoagulation for the mechanical prothesis. The construction of a tissue engineered living heart valve could eliminate these problems.

Atherosclerosis and cardiovascular disease are also major causes of morbidity and mortality. More than 925,000 Americans died from heart and blood vessels disease in 1992, and an estimated 468,000 coronary artery bypass surgeries were performed on 393,000 patients. This does not include bypass procedures for peripheral vascular disease. Currently, internal mammary and saphenous vein grafts are the most frequently used native grafts for coronary bypass surgery. However, with triple and quadruple bypasses and often the need for repeat bypass procedures, sufficient native vein grafts can be a problem. Surgeons must frequently look for vessels other than the internal mammary and saphenous vessels. While large diameter (0.5 mm internal diameter) vascular grafts of dacron or polytetraflorethylene (PTFE) have been successful, small caliber synthetic vascular grafts frequently do not remain patent over time. Tissue engineered blood vessels may offer a substitute for small caliber vessels for bypass surgery and replacement of diseased vessels.

It is therefore an object of the present invention to provide a method for making tissue engineered constructs which have improved mechanical strength and flexibility.

It is a further object of the present invention to provide a method and materials for making valves and vessels which can withstand repeated stress and strain.

It is another object of the present invention to provide a method improving yields of engineered tissues following implantation.

SUMMARY OF THE INVENTION

It has been discovered that improved yields of engineered tissue following implantation, and engineered tissue having enhanced mechanical strength and flexibility or pliability, can be obtained by implantation, preferably subcutaneously, of a fibrous polymeric matrix for a period of time sufficient to obtain ingrowth of fibrous tissue and/or blood vessels, which is then removed for subsequent implantation at the site where the implant is desired. The matrix is optionally seeded prior to the first implantation, after ingrowth of the fibrous tissue, or at the time of reimplantation. The time required for fibrous ingrowth typically ranges from days to weeks. The method is particularly useful in making valves and tubular structures, especially heart valves and blood vessels.

Examples demonstrate construction of blood vessels, heart valves and bone and cartilage composite structures.

DETAILED DESCRIPTION OF THE INVENTION

As described herein, structures are created by seeding of fibrous or porous polymeric matrices with dissociated cells which are useful for a variety of applications, ranging from

soft tissues formed of parenchymal cells such as hepatocytes, to tissues having structural elements such as heart valves and blood vessels, to cartilage and bone. In a particular improvement over the prior art methods, the polymeric matrices are implanted into a human or animal to allow ingrowth of fibroblastic tissue, then implanted at the site where the structure is needed, either alone or seeded with defined cell populations.

I. Matrix Fabrication

The synthetic matrix serves several purposes. It functions as a cell delivery system that enables the organized transplantation of large numbers of cells into the body. The matrix acts as a scaffold providing three-dimensional space for cell growth. The matrix functions as a template providing structural cues for tissue development. In the case of tissues have specific requirements for structure and mechanical strength, the polymer temporarily provides the biomechanical properties of the final construct, giving the cells time to lay down their own extracellular matrix which ultimately is responsible for the biomechanical profile of the construct. The scaffold also determines the limits of tissue growth and thereby determines the ultimate shape of tissue engineered construct. Cells implanted on a matrix proliferate only to the edges of the matrix; not beyond.

Matrix Architecture

As previously described, for a tissue to be constructed, successfully implanted, and function, the matrices must have sufficient surface area and exposure to nutrients such that cellular growth and differentiation can occur prior to the ingrowth of blood vessels following implantation. This is not a limiting feature where the matrix is implanted and ingrowth of tissue from the body occurs, prior to seeding of the matrix with dissociated cells.

The organization of the tissue may be regulated by the microstructure of the matrix. Specific pore sizes and structures may be utilized to control the pattern and extent of fibrovascular tissue ingrowth from the host, as well as the organization of the implanted cells. The surface geometry and chemistry of the matrix may be regulated to control the adhesion, organization, and function of implanted cells or host cells.

In the preferred embodiment, the matrix is formed of polymers having a fibrous structure which has sufficient interstitial spacing to allow for free diffusion of nutrients and gases to cells attached to the matrix surface. This spacing is typically in the range of 100 to 300 microns, although closer spacings can be used if the matrix is implanted, blood vessels allowed to infiltrate the matrix, then the cells are seeded into the matrix. As used herein, "fibrous" includes one or more fibers that is entwined with itself, multiple fibers in a woven or non-woven mesh, and sponge like devices.

The matrix should be a pliable, non-toxic, injectable porous template for vascular ingrowth. The pores should allow vascular ingrowth and the injection of cells in a desired density and region(s) of the matrix without damage to the cells. These are generally interconnected pores in the range of between approximately 100 and 300 microns. The matrix should be shaped to maximize surface area, to allow adequate diffusion of nutrients and growth factors to the cells and to allow the ingrowth of new blood vessels and connective tissue.

The overall, or external, matrix configuration is dependent on the tissue which is to reconstructed or augmented. The shape can also be obtained using struts, as described below, to impart resistance to mechanical forces and thereby yield the desired shape. Examples include heart valve "leaflets" and tubes.

Polymers

The term "bioerodible", or "biodegradable", as used herein refers to materials which are enzymatically or chemically degraded in vivo into simpler chemical species. Either natural or synthetic polymers can be used to form the matrix, although synthetic biodegradable polymers are preferred for reproducibility and controlled release kinetics. Synthetic polymers that can be used include bioerodible polymers such as poly(lactide) (PLA), poly(glycolic acid) (PGA), poly(lactide-co-glycolide) (PLGA), poly(caprolactone), polycarbonates, polyamides, polyanhydrides, polyamino acids, polyortho esters, polyacetals, polycyanoacrylates and degradable polyurethanes, and non-erodible polymers such as polyacrylates, ethylene-vinyl acetate polymers and other acyl substituted cellulose acetates and derivatives thereof, non-erodible polyurethanes, polystyrenes, polyvinyl chloride, polyvinyl fluoride, poly(vinyl imidazole), chlorosulphonated polyolifins, polyethylene oxide, polyvinyl alcohol, teflon®, and nylon. Although non-degradable materials can be used to form the matrix or a portion of the matrix, they are not preferred. The preferred non-degradable material for implantation of a matrix which is prevascularized prior to implantation of dissociated cells is a polyvinyl alcohol sponge, or alkylation, and acylation derivatives thereof, including esters. A non-absorbable polyvinyl alcohol sponge is available commercially as Ivalon™, from Unipoint Industries. Methods for making this material are described in U.S. Pat. Nos. 2,609,347 to Wilson; 2,653,917 to Hammon, 2,659,935 to Hammon, 2,664,366 to Wilson, 2,664,367 to Wilson, and 2,846,407 to Wilson, the teachings of which are incorporated by reference herein. These materials are all commercially available.

Examples of natural polymers include proteins such as albumin, collagen, synthetic polyamino acids, and prolamines, and polysaccharides such as alginate, heparin, and other naturally occurring biodegradable polymers of sugar units. These are not preferred because of difficulty with quality control and lack of reproducible, defined degradation characteristics.

PLA, PGA and PLA/PGA copolymers are particularly useful for forming the biodegradable matrices. These are synthetic, biodegradable α-hydroxy acids with a long history of medical use. PLA polymers are usually prepared from the cyclic esters of lactic acids. Both L(+) and D(−) forms of lactic acid can be used to prepare the PLA polymers, as well as the optically inactive DL-lactie acid mixture of D(−) and L(+) lactic aeids. Methods of preparing polylactides are well documented in the patent literature. The following U.S. Patents, the teachings of which are hereby incorporated by reference, describe in detail suitable polylactides, their properties and their preparation: U.S. Pat. Nos. 1,995,970 to Dorough; 2,703,316 to Schneider; 2,758,987 to Salzberg; 2,951,828 to Zeile; 2,676,945 to Higgins; and 2,683,136; 3,531,561 to Trehu.

PGA is the homopolymer of glycolic acid (hydroxyacetic acid). In the conversion of glycolic acid to poly(glycolic acid), glycolic acid is initially reacted with itself to form the cyclic ester glycolide, which in the presence of heat and a catalyst is converted to a high molecular weight linear-chain polymer. PGA polymers and their properties are described in more detail in Cyanamid Research Develops World's First Synthetic Absorbable Suture", Chemistry and Industry, 905 (1970).

The erosion of the matrix is related to the molecular weights of the polymer, for example, PLA, PGA or PLA/PGA. The higher molecular weights, weight average

molecular weights of 90,000 or higher, result in polymer matrices which retain their structural integrity for longer periods of time; while lower molecular weights, weight average molecular weights of 30,000 or less, result in both slower release and shorter matrix lives. A preferred material is poly(lactide-co-glycolide) (50:50), which degrades in about six weeks following implantation (between one and two months) and poly(glycolic acid).

All polymers for use in the matrix must meet the mechanical and biochemical parameters necessary to provide adequate support for the cells with subsequent growth and proliferation. The polymers can be characterized with respect to mechanical properties such as tensile strength using an Instron tester, for polymer molecular weight by gel permeation chromatography (GPC), glass transition temperature by differential scanning calorimetry (DSC) and bond structure by infrared (IR) spectroscopy, with respect to toxicology by initial screening tests involving Ames assays and in vitro teratogenicity assays, and implantation studies in animals for immunogenicity, inflammation, release and degradation studies.

Polymer Coatings

In some embodiments, attachment of the cells to the polymer is enhanced by coating the polymers with compounds such as basement membrane components, agar, agarose, gelatin, gum arabic, collagens types I, II, III, IV, and V, fibronectin, laminin, glycosaminoglycans, polyvinyl alcohol, mixtures thereof, and other hydrophilic and peptide attachment materials known to those skilled in the art of cell culture. A preferred material for coating the polymeric matrix is polyvinyl alcohol or collagen.

Struts

In some embodiments it may be desirable to create additional structure using devices provided for support, referred to herein as "struts". These can be biodegradable or non-degradable polymers which are inserted to form a more defined shape than is obtained using the cell-matrices. An analogy can be made to a corset, with the struts acting as "stays" to push the surrounding tissue and skin up and away from the implanted cells. In a preferred embodiment, the struts are implanted prior to or at the time of implantation of the cell-matrix structure. The struts are formed of a polymeric material of the same type as can be used to form the matrix, as listed above, having sufficient strength to resist the necessary mechanical forces.

Additives to Polymer Matrices

In some embodiments it may be desirable to add bioactive molecules to the cells. A variety of bioactive molecules can be delivered using the matrices described herein. These are referred to generically herein as "factors" or "bioactive factors".

In the preferred embodiment, the bioactive factors are growth factors, angiogenic factors, compounds selectively inhibiting ingrowth of fibroblast tissue such as antiinflammatories, and compounds selectively inhibiting growth and proliferation of transformed (cancerous) cells. These factors may be utilized to control the growth and function of implanted cells, the ingrowth of blood vessels into the forming tissue, and/or the deposition and organization of fibrous tissue around the implant.

Examples of growth factors include heparin binding growth factor (hbgf), transforming growth factor alpha or beta (TGFβ), alpha fibroblastic growth factor (FGF), epidermal growth factor (TGF), vascular endothelium growth factor (VEGF), some of which are also angiogenic factors. Other factors include hormones such as insulin, glucagon, and estrogen. In some embodiments it may be desirable to incorporate factors such as nerve growth factor (NGF) or muscle morphogenic factor (MMP).

Steroidal antiünflammatories can be used to decrease inflammation to the implanted matrix, thereby decreasing the amount of fibroblast tissue growing into the matrix.

These factors are known to those skilled in the art and are available commercially or described in the literature. In vivo dosages are calculated based on in vitro release studies in cell culture; an effective dosage is that dosage which increases cell proliferation or survival as compared with controls, as described in more detail in the following examples. Preferably, the bioactive factors are incorporated to between one and 30% by weight, although the factors can be incorporated to a weight percentage between 0.01 and 95 weight percentage.

Bioactive molecules can be incorporated into the matrix and released over time by diffusion and/or degradation of the matrix, they can be suspended with the cell suspension, they can be incorporated into microspheres which are suspended with the cells or attached to or incorporated within the matrix, or some combination thereof. Microspheres would typically be formed of materials similar to those forming the matrix, selected for their release properties rather than structural properties. Release properties can also be determined by the size and physical characteristics of the microspheres.

II. Cells to Be Implanted

Cells to be implanted are dissociated using standard techniques such as digestion with a collagenase, trypsin or other protease solution. Preferred cell types are mesenchymal cells, especially smooth or skeletal muscle cells, myocytes (muscle stem cells), fibroblasts, chondrocytes, adipocytes, fibromyoblasts, and ectodermal cells, including ductile and skin cells, hepatocytes, Islet cells, cells present in the intestine, and other parenchymal cells, osteoblasts and other cells forming bone or cartilage. In some cases it may also be desirable to include nerve cells. Cells can be normal or genetically engineered to provide additional or normal function. Methods for genetically engineering cells with retroviral vectors, polyethylene glycol, or other methods known to those skilled in the art can be used.

Cells are preferably autologous cells, obtained by biopsy and expanded in culture, although cells from close relatives or other donors of the same species may be used with appropriate immunosuppression. Immunologically inert cells, such as embryonic or fetal cells, stem cells, and cells genetically engineered to avoid the need for immunosuppression can also be used. Methods and drugs for immunosuppression are known to those skilled in the art of transplantation. A preferred compound is cyclosporin using the recommended dosages.

In the preferred embodiment, cells are obtained by biopsy and expanded in culture for subsequent implantation. Cells can be easily obtained through a biopsy anywhere in the body, for example, skeletal muscle biopsies can be obtained easily from the arm, forearm, or lower extremities, and smooth muscle can be obtained from the area adjacent to the subcutaneous tissue throughout the body. To obtain either type of muscle, the area to be biopsied can be locally anesthetized with a small amount of lidocaine injected subcutaneously. Alternatively, a small patch of lidocaine jelly can be applied over the area to be biopsied and left in

place for a period of 5 to 20 minutes, prior to obtaining biopsy specimen. The biopsy can be effortlessly obtained with the use of a biopsy needle, a rapid action needle which makes the procedure extremely simple and almost painless. With the addition of the anesthetic agent, the procedure would be entirely painless. This small biopsy core of either skeletal or smooth muscle can then be transferred to media consisting of phosphate buffered saline. The biopsy specimen is then transferred to the lab where the muscle can be grown utilizing the explant technique, wherein the muscle is divided into very pieces which are adhered to culture plate, and serum containing media is added. Alternatively, the muscle biopsy can be enzymatically digested with agents such as trypsin and the cells dispersed in a culture plate with any of the routinely used medias. After cell expansion within the culture plate, the cells can be easily passaged utilizing the usual technique until an adequate number of cell is achieved.

III. Methods for Implantation

Unlike other prior art methods for making implantable matrices, the present method uses the recipient or an animal as the initial bioreactor to form a fibrous tissue-polymeric construct which optionally can be seeded with other cells and implanted. The matrix becomes infiltrated with fibrous tissue and/or blood vessels over a period ranging from between one day and a few weeks, most preferably one and two weeks. The matrix is then removed and implanted at the site where it is needed.

In one embodiment, the matrix is formed of polymer fibers having a particular desired shape, that is implanted subcutaneously. The implant is retrieved surgically, then one or more defined cell types distributed onto and into the fibers. In a second embodiment, the matrix is seeded with cells of a defined type, implanted until fibrous tissue has grown into the matrix, then the matrix removed, optionally cultured further in vitro, then reimplanted at a desired site.

The resulting structures are dictated by the matrix construction, including architecture, porosity (% void volume and pore diameter), polymer nature including composition, crystallinity, molecular weight, and degradability, hydrophobicity, and the inclusion of other biologically active molecules.

This methodology is particularly well suited for the construction of valves and tubular structures. Examples of valves are heart valves and valves of the type used for ventricular shunts for treatment of hydrocephaly. A similar structure could be used for an ascites shunt in the abdomen where needed due to liver disease or in the case of a lymphatic obstructive disease. Examples of tubular structures include blood vessels, intestine, ureters, and fallopian tubes.

The structures are formed at a site other than where they are ultimately required. This is particularly important in the case of tubular structures and valves, where integrity to fluid is essential, and where the structure is subjected to repeated stress and strain.

The present invention will be further understood by reference to the following non-limiting examples.

EXAMPLE 1

TISSUE ENGINEERING OF HEART VALVES

Valvular heart disease is a significant cause of morbidity and mortality. Construction of a tissue engineered valve using living autologous cells offers advantages over currently used mechanical or glutaraldehyde fixed xenograft valves.

Methods and Materials

A tissue engineered valve was constructed by seeding a synthetic polyglycolic acid (PGA) fiber based matrix with dissociated fibroblasts and endothelial cells harvested from a donor sheep heart valve. The cells were grown to confluence and split several times to increase the cell number. A mixed cell population including myofibroblasts and endothelial cells was obtained. The endothelial cells were labeled with an Ac-Dil-LDL fluorescent antibody obtained from a commercial source and sorted in a cell-sorting machine to yield a nearly pure endothelial cell population (LDL+) and a mixed cell population containing myofibroblasts and endothelial cells (LDL−). A PGA mesh (density 76.9 mg/ml and thickness 0.68 mm) was seeded with the mixed cell population and grown in culture. When the myofibroblasts reached confluence, endothelial cells were seeded onto the surface of the fibroblast/mesh constructs and grown into a single monolayer.

Immunohistochemical evaluation of constructs with antibodies against factor VIII, a specific marker for endothelial cells, revealed that tissue engineered valves histologically resemble native valve tissue. The effects of physiological flow on elastin and collagen production within the ECM were examined in a bioreactor and implanted in a sheep to determine if the constructs had the required pliability and mechanical strength for use in patients.

EXAMPLE 2

TISSUE ENGINEERING OF VASCULAR STRUCTURES

Vascular smooth muscle tubular structures using a biodegradable polyglycolic acid polymer scaffold have been developed. The technique involves the isolation and culture of vascular smooth muscle cells, the reconstruction of a vascular wall using biodegradable polymer, and formation of the neo-tissue tubes in vitro. The feasibility of engineering vascular structures by coculturing endothelial cells with fibroblasts and smooth muscle cells on a synthetic biodegradable matrix in order to create tubular constructs which histologically resemble native vascular structures was also demonstrated.

Methods

In a first set of studies, bovine and ovine endothelial cells, smooth muscle cells, and fibroblasts were isolated using a combination of standard techniques including collagenase digestion and explantation. These cells were then expanded in tissue culture. All cells were grown in Delbecco's modified Eagle's media supplemented with 10% fetal bovine serum, 1% antibiotic solution, and basic fibroblast growth factor. Mixed colonies were purified using dilutional cloning. Thirty (N=30) two by two centimeter polyglycolic acid (PGA) fiber meshes (thickness=0.68 mm, density=76.9 mg/cc) were then serially seeded with 5×10^5 fibroblasts and smooth muscle cells and placed in culture. Five (N=5) 85% PGA, 15% polylatic acid tubular constructs (length=2 cm, diameter=0.8 cm) were seeded in a similar fashion. After the fibroblasts and smooth muscle cell constructs had grown to confluence (mean time 3 weeks), 1×10^6 endothelial cells were seeded onto them and they were placed in culture for one week. These vascular constructs were then fixed in a paraffin, sectioned and analyzed using immunohistochemical staining for factor VIII (specific for endothelial cells) and desmin (specific for muscle cells).

In a second set of studies, smooth muscle cells were obtained by harvesting the media from the artery of a lamb using standard explant techniques. Cells were expanded in culture through repeated passages and then seeded on the biodegradable polymer scaffold at a density of 1×10^6 cells per cm^2 of polymer. The cell-polymer constructs were formed into tubes with internal diameters ranging from 2 mm to 5 mm and maintained in vitro for 6 to 8 weeks.

Results

Microscopic examination of all constructs in the first study (N=30/N=5) revealed that both types of constructs had achieved the proper histological architecture and resembled native vessels after one week. Immunohistochemical staining confirmed that endothelially lined smooth muscle/fibroblast tubes had been created. The extracellular matrices (ECM) of the vascular constructs were examined in order to determine the composition of elastin and collagen types I and III, the ECM molecules which determine the physical characteristics of native vascular tissues.

The results of the second study show that vascular smooth muscle tubes which retain their structure can be successfully formed using a polyglycolic acid polymer scaffold. The biodegradable polymer was absorbed over time, leaving a neo-tissue vascular smooth muscle tube.

EXAMPLE 3

ENGINEERED BONE FROM PGA POLYMER SCAFFOLD AND PERIOSTEUM

The ability to create bone from periosteum and biodegradable polymer may have significant utility in reconstructive orthopedic and plastic surgery. Polyglycolic acid (PGA) is a preferred material for forming a biodegradable matrix which can be configured to a desirable shape and structure. This study was conducted to determine whether new bone constructs can be formed from periosteum or periosteal cells placed onto PGA polymer.

Materials and Methods

Bovine periosteum, harvested from fresh calf limbs, was placed either directly onto PGA polymer (1×1 cm) or onto tissue culture dishes for periosteal cell isolation. The periosteum/PGA construct was cultured for one week in MEM 199 culture media with antibiotics and ascorbic acid, then implanted into the dorsal subcutaneous space of nude mice. Periosteal cell, cultured from pieces of periosteum for two weeks, were isolated into cell suspension and seeded (approximately 1 to 3×10^7 cells) onto PGA polymer (1×1 cm); after one week in culture, the periosteal cell seeded polymer was implanted into the subcutaneous space of nude mice. Specimens, harvested at 4, 8, and 14 week intervals, were evaluated grossly and histologically.

Results

The periosteum/PGA constructs showed an organized cartilage matrix with early evidence of bone formation at four weeks, a mixture of bone and cartilage at 8 weeks, and a complete bone matrix at 14 weeks. Constructs created from periosteal cells seeded onto polymer showed presence of disorganized cartilage at 4 and 8 weeks, and a mixture of bone and cartilage at 14 weeks. Periosteum placed directly onto polymer will form an organized cartilage and bone matrix earlier than constructs formed from periosteal cell seeded polymer. This data indicates that PGA is an effective scaffold for periosteal cell attachment and migration to produce bone, which may offer new approaches to reconstructive surgery.

EXAMPLE 4

BONE RECONSTRUCTION WITH TISSUE ENGINEERING VASCULARIZED BONE

The aim of this study was to determine if new vascularized bone could be engineered by transplantation of osteoblast around existing vascular pedicle using biodegradable polymers as cell delivery devices, to be used to reconstruct weight bearing bony defects.

Methods

Osteoblast and chondryocytes were isolated from calf periosteum and articular cartilage, cultured in vitro for three weeks, then seeded onto a 1×1 cm non-woven polyglycolic acid (PGA) mesh. After maintenance in vitro for one week, cell-polymer constructs were wrapped around saphenous vessels, and implanted into athymic rats for 8 weeks. The implants showed gross and histological evidence of vascularized bone or cartilage. At this time, bilateral 0.8 cm femoral shaft defect were created in the same rat, and fixed in position with a 3 cm craniofacial titanium miniplate. The new engineered bone/cartilage construct was then transferred to the femoral defect on its bilateral vascular pedicle. A total of 30 femoral defects were repaired in three groups of animals (each group composed of five animals with defects). Animals in Group 1 received implants composed of vascularized bone constructs, animals in Group 2 with vascular cartilage constructs, and Group 3 animals with blank polymer only.

At six months after surgery, the animals were studied radiographically for evidence of new bone formation at the site of the defect. Euthanasia was then performed by anesthetic overdose and each experimented femur was removed. Gross appearance was recorded and histological studies performed using hematoxylin and eosin (H & E) staining.

Results

Group 1 defect showed evidence of new bone formation around the defect. Neither Group 2 nor Group 3 defect showed any radiographic evidence of healing or bone formation. Grossly, Group 1 animals developed exuberant bony callus formation and healing of the defect. The animals in Group 2 showed filling of the bony defect with cartilaginous tissue, whereas all of the animals in Group 3 either developed a fibrous non-union or simple separation of both bony fragments with soft tissue invasion of the defect. The histological studies showed new bone formation in all Group 1 animals, new cartilage formation in all Group 2 animals, and fibrous tissue invasion in all Group 3 animals.

Conclusion

In conclusion, it was possible to engineer vascularized bone and cartilage grafts, which could be used to repair bone defects in the rat femur. Engineered tissue maintained the characteristics of the tissues form which the cells were originally isolated.

EXAMPLE 5

ENGINEERING OF COMPOSITE BONE AND CARTILAGE

The ability to construct a composite structure of bone and cartilage offers a significant modality in reconstructive plastic and orthopedic surgery. The following study was conducted to engineer a bone and cartilage composite structure using periosteum, chondrocytes and biodegradable polymer and to direct bone and cartilage formation by selectively placing periosteum and chondrocytes onto the polymer scaffold.

Methods and materials

Bovine periosteum and cartilage were harvested from newborn calf limbs. Periosteum (1.5×2.0 cm) was wrapped around a polyglycolic acid/poly L-lactic acid co-polymer tube (3 cm in length, 3 mm in diameter), leaving the ends exposed. The cartilage pieces were enzymatically digested with collagenase, and chondrocytes (2×10^7 cells) were seeded onto each end of the exposed polymer. The composite construct was cultured for seven days in Medium 199 with antibiotics, fetal bovine serum, and ascorbic acid at 37° C. with 5% CO_2. Eight constructs were then implanted into the dorsal subcutaneous space of eight nude mice. After 8 to 14 weeks in vivo, the implants were harvested and evaluated grossly and histologically.

Results

All implants formed into cylindrical shapes, flattened at the ends. The central portion of the implant formed into a bony matrix and the ends of the specimens formed into cartilage, approximately where the periosteum and chondrocytes were placed. Histological sections showed an organized matrix of bone and cartilage with a distinct transition between bone and cartilage.

Conclusions

The results show that periosteum and chondrocytes placed onto a biodegradable polymer will form into a composite tissue of bone and cartilage. Moreoever, bone and cartilage composite formation with selective placement of periosteum and chondrocytes on a biodegradable polymer scaffold was shown.

EXAMPLE 6

IMPLANTATION OF MATRIX FOR INGROWTH OF FIBROUS TISSUE TO INCREASE MECHANICAL PROPERTIES AND CELL SURVIVAL

The following study was conducted to increase the mechanical strength and pliability of the heart valve leaflets or other engineered tissues such as those for use as blood vessels.

Methods

A PGA mesh as described in Example 1 or 2 was implanted subcutaneously in an animal, then removed after a period of one to two weeks. Fibroblasts migrated into the polymeric mesh while it was implanted. The implant was then seeded with other cells such as chondrocytes or endothelial cells and cultured in vitro for an additional period of time.

Results

The resulting implant was shown to have greater mechanical strength and pliability than implants formed solely by seeding of dissociated cells.

Modifications and variations of the method and compositions described herein will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the appended claims.

We claim:

1. A cell-matrix structure comprising

a fibrous matrix formed of a biocompatible, biodegradable synthetic polymer, and

seeded with dissociated human cells,

wherein the matrix is configured to form a tissue structure having mechanical strength and flexibility or pliability,

wherein the cell-matrix structure is formed by seeding the matrix, implanting the seeded matrix into a recipient human or animal for a period of time sufficient to form extracellular matrix, and harvesting of the resulting cell-matrix structure.

2. The cell-matrix structure of claim **1** wherein the matrix is configured to form a tube.

3. The cell-matrix structure of claim **1** wherein the matrix is configured to form a valve in a blood vessel, intestine, or heart.

4. The cell-matrix structure of claim **3** wherein the matrix is configured to form a heart valve.

5. The cell-matrix structure of claim **1** wherein the cells are selected from the group of consisting of parenchymal and connective tissue cells.

6. A tissue-engineered heart valve formed of a porous polymeric matrix seeded with dissociated endothelial and fibroblast cells, wherein the cells form extracellular matrix following implantation into a human or animal recipient, and wherein the extracellular matrix is shaped to form a heart valve.

7. The heart valve of claim **6** wherein the matrix is formed of a polymer selected from the group consisting of poly (lactic acid), poly(glycolic acid), and combinations thereof.

8. The heart valve of claim **7** wherein the matrix is formed of polymer fibers having an interstitial spacing of between 100 and 300 microns and having pore sizes and structure to control the pattern and extent of fibroblastic tissue ingrowth following implantation.

9. The heart valve of claim **7** wherein the matrix was seeded with dissociated cells selected from the group consisting of fibroblasts, myofibroblasts, and endothelial cells and includes elastin fibers.

* * * * *

US006534084B1

(12) **United States Patent**
Vyakarnam et al.

(10) **Patent No.: US 6,534,084 B1**
(45) **Date of Patent: Mar. 18, 2003**

(54) **POROUS TISSUE SCAFFOLDINGS FOR THE REPAIR OR REGENERATION OF TISSUE**

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(73) Assignee: **Ethicon, Inc.**, Somerville, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 301 days.

(21) Appl. No.: **09/740,289**

(22) Filed: **Dec. 19, 2000**

Related U.S. Application Data

(62) Division of application No. 09/345,096, filed on Jun. 30, 1999.

(51) **Int. Cl.**[7] **A61F 13/00**

(52) **U.S. Cl.** **424/443**; 424/424; 424/425; 424/426; 424/444; 424/446; 424/447; 514/2; 514/772.3; 514/945; 514/93.1

(58) **Field of Search** 424/424, 425, 424/426, 443, 444, 446, 447, 93.1; 514/2, 772.3, 945

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Primary Examiner—Samuel A. Acquah

(57) **ABSTRACT**

The present patent describes a three-dimensional interconnected open cell porous foams that have a gradient in composition and/or microstructure through one or more directions. These foams can be made from a blend of absorbable and biocompatible polymers that are formed into foams having a compositional gradient transitioning from predominately one polymeric material to predominately a second polymeric material. These gradient foams are particularly well suited to tissue engineering applications and can be designed to mimic tissue transition or interface zones.

36 Claims, 14 Drawing Sheets

(2 of 14 Drawing Sheet(s) Filed in Color)

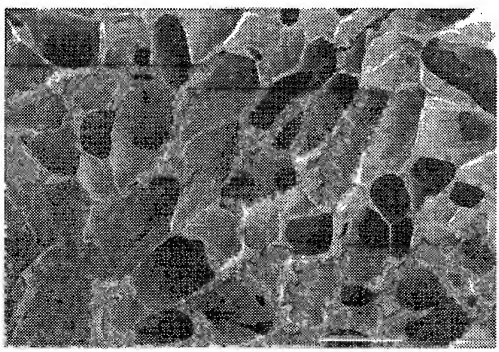


FIG. 1

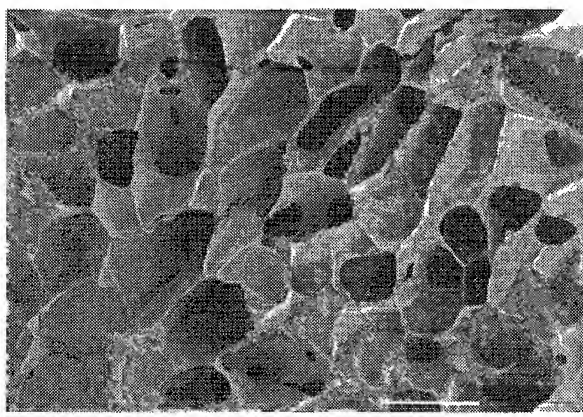


FIG. 2

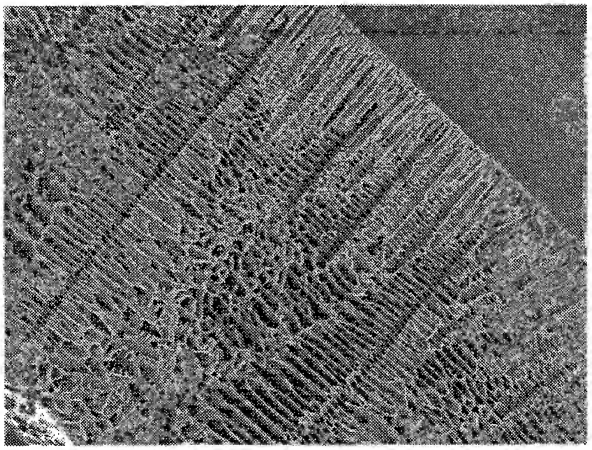


FIG. 3

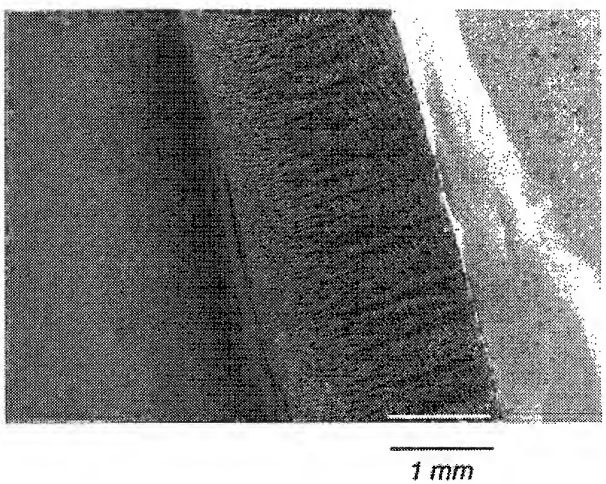


FIG. 4

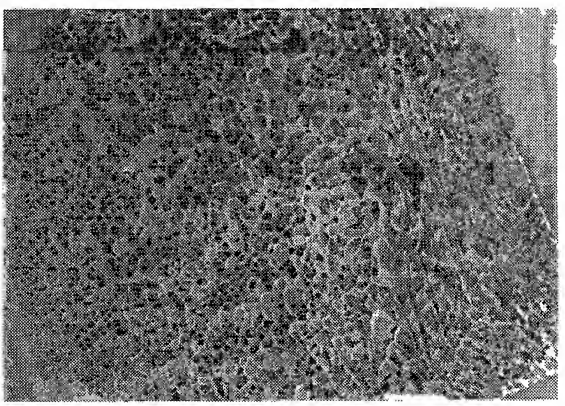


FIG. 5

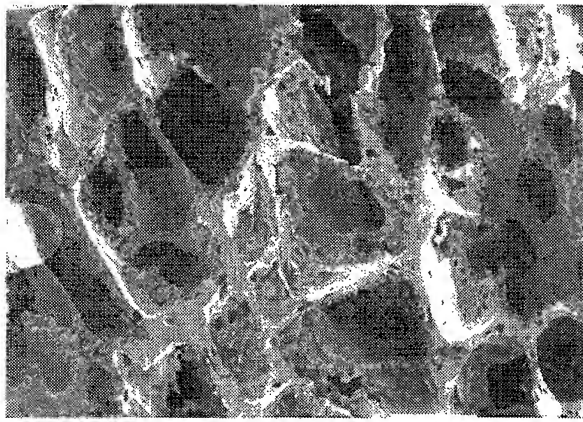


FIG. 6

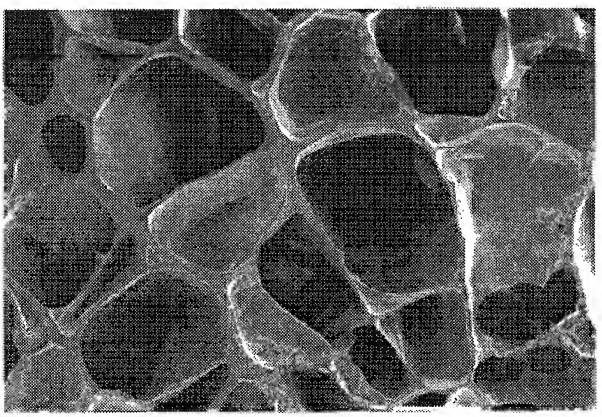


FIG. 7A

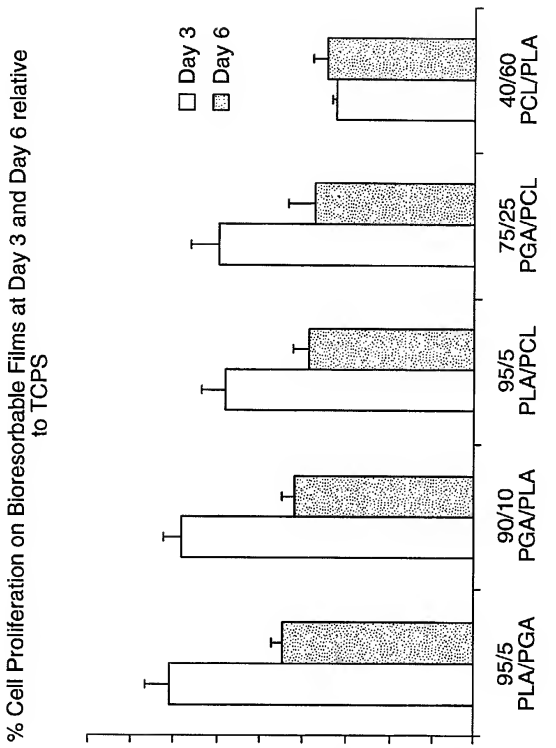

% Cell Proliferation on Bioresorbable Films at Day 3 and Day 6 relative to TCPS
% Cell Proliferation
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Day 3
Day 6
95/5 PLA/PGA
90/10 PGA/PLA
95/5 PLA/PCL
75/25 PGA/PCL
40/60 PCL/PLA

FIG. 7B

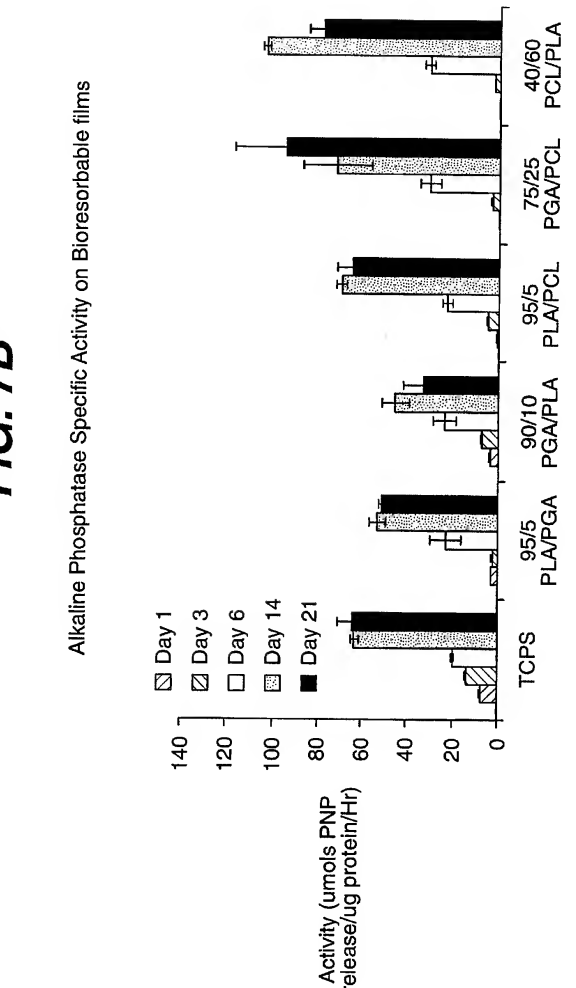

Alkaline Phosphatase Specific Activity on Bioresorbable films
Activity (umols PNP release/ug protein/Hr)
0
20
40
60
80
100
120
140
Day 1
Day 3
Day 6
Day 14
Day 21
TCPS
95/5 PLA/PGA
90/10 PGA/PLA
95/5 PLA/PCL
75/25 PGA/PCL
40/60 PCL/PLA

FIG. 7C

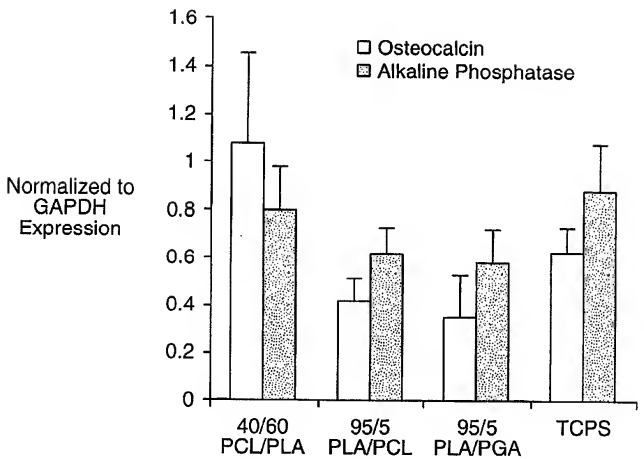

Normalized to GAPDH Expression
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Osteocalcin
Alkaline Phosphatase
40/60 PCL/PLA
95/5 PLA/PCL
95/5 PLA/PGA
TCPS

FIG. 8

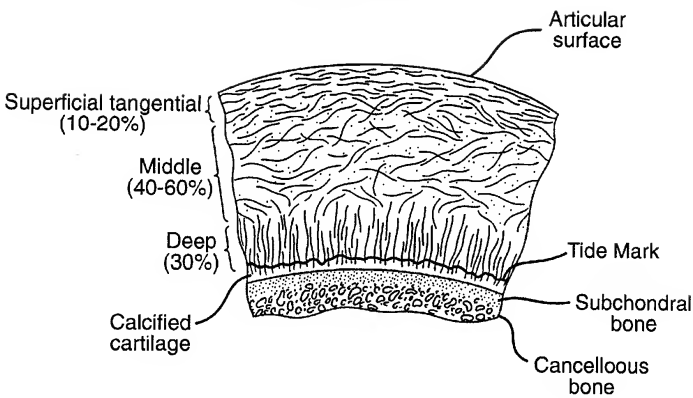

Articular surface
Superficial tangential (10-20%)
Middle (40-60%)
Deep (30%)
Tide Mark
Subchondral bone
Calcified cartilage
Cancelloous bone

FIG. 9A

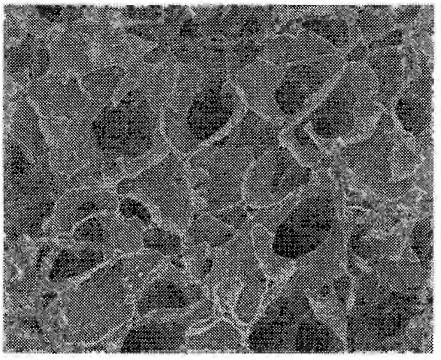


FIG. 9B

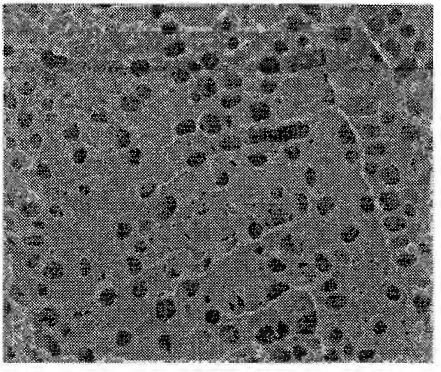


FIG. 9C

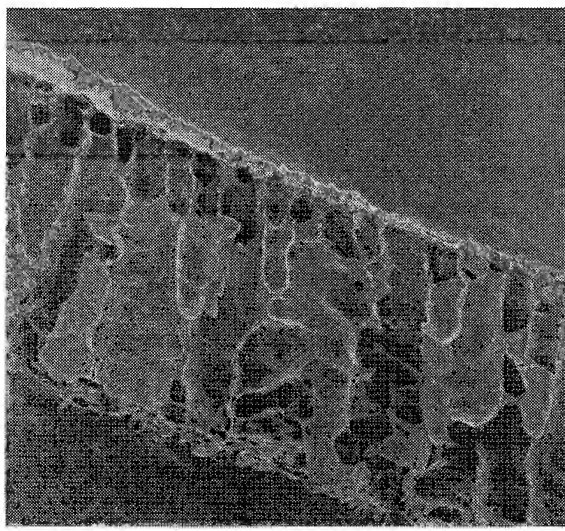


FIG. 10

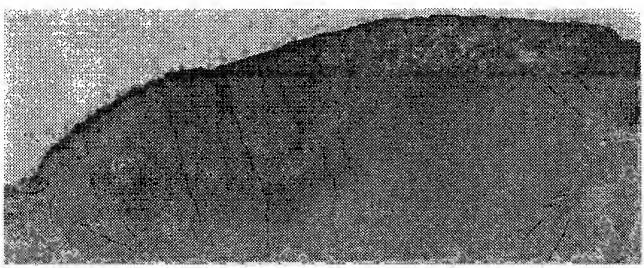
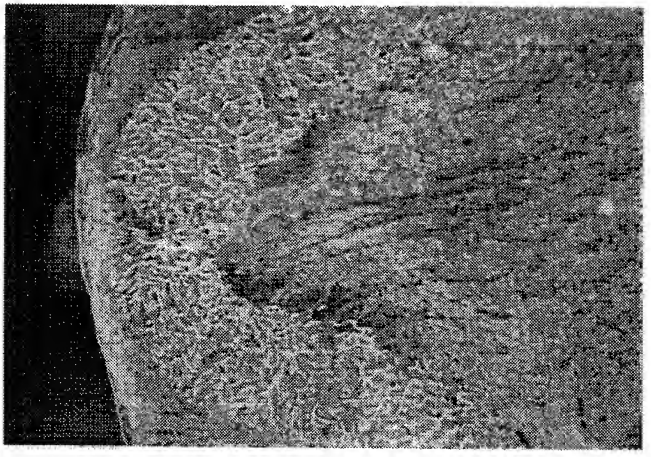


FIG. 11



POROUS TISSUE SCAFFOLDINGS FOR THE REPAIR OR REGENERATION OF TISSUE

This appln is a Div. of Ser. No. 09/345,096 filed Jun. 30, 1999.

FIELD OF THE INVENTION

The present invention relates generally to the field of tissue repair and regeneration. More particularly the present invention relates to porous biocompatible bioabsbrbable foams that have a gradient in composition and/or microstructure that serve as a template for tissue regeneration, repair or augmentation.

BACKGROUND OF THE INVENTION

Open cell porous biocompatible foams have been recognized to have significant potential for use in the repair and regeneration of tissue. Early efforts in tissue repair focused on the use of amorphous biocompatible foam as porous plugs to fill voids in bone. Brekke, et al. (U.S. Pat. No. 4,186,448) described the use of porous mesh plugs composed of polyhydroxy acid polymers such as polylactide for healing bone voids. Several attempts have been made in the recent past to make TE scaffolds using different methods, for example U.S. Pat. Nos. 5,522,895 (Mikos) and 5,514,378 (Mikos, et al.) using leachables; U.S. Pat. Nos. 5,755,792 (Brekke) and 5,133,755 (Brekke) using vacuum foaming techniques; U.S. Pat. Nos. 5,716,413 (Walter, et al.) and 5,607,474 (Athanasiou, et al.) using precipitated polymer gel masses; U.S. Pat. Nos. 5,686,091 (Leong, et al.) and 5,677, 355 (Shalaby, et al.) using polymer melts with fugitive compounds that sublimate at temperatures greater than room temperature; and U.S. Pat. Nos. 5,770,193 (Vacanti, et al.) 5,769,899 (Schwartz, et al.) and 5,711,960 (Shikinami) using textile-based fibrous scaffolds. Hinsch et al. (EPA 274,898) described a porous open cell foam of polyhydroxy acids with pore sizes from about 10 to about 200 μm for the in-growth of blood vessels and cells. The foam described by Hincsh could also be reinforced with fibers, yarns, braids, knitted fabrics, scrims and the like. Hincsh's work also described the use of a variety of polyhydroxy acid polymers and copolymers such as poly-L-lactide, poly-DL-lactide, polyglycolide, and polydioxanone. The Hincsh foams had the advantage of having regular pore sizes and shapes that could be controlled by the processing conditions, solvents selected, and the additives.

However, the above techniques have limitations in producing a scaffold with a gradient structure. Most of the scaffolds are isotropic in form and function and lack the anisotropic features of natural tissues.

Further, it is the limitation of prior art to make 3D scaffolds that have the ability to control the spatial distribution of various pore shapes. The process that is described to fabricate the microstructure controlled foams is a low temperature process that offers many advantages over other conventional techniques. For example the process allows the incorporation of thermally sensitive compounds like proteins, drugs and other additives with the thermally and hydrolytically unstable absorbable polymers.

Athanasiou et al. (U.S. Pat. No. 5,607,474) have more recently proposed using a two layer foam device for repairing osteochondral defects at a location where two dissimilar types of tissue are present. The Athanasiou device is composed of a first and second layer, prepared in part separately, and joined together at a subsequent step. Each of the scaffold layers is designed to have stiffness and compressibility

corresponding to the respective cartilage and bone tissue. Since cartilage and bone often form adjacent layers in the body this approach is an attempt to more clearly mimic the structure of the human body. However, the interface between the cartilage and bone in the human body is not a discrete junction-of-two dissimilar materials with an abrupt change in anatomical features and/or the mechanical properties. The cartilage cells have distinctly different cell morphology and orientation depending on the location of the cartilage cell in relation to the underlying bone structure. The difference in cartilage cell morphology and orientation provides a continuous transition from the outer surface of the cartilage to the underlying bone cartilage interface. Thus the two layer system of Athanasiou, although an incremental improvement, does not mimic the tissue interfaces present in the human body.

Another approach to make three-dimensional laminated foams is proposed by Mikos et al. (U.S. Pat. No. 5,514,378). In this technique which is quite cumbersome, a porous membrane is first prepared by drying a polymer solution containing leachable salt crystals. A three-dimensional structure is then obtained by laminating several membranes together, which are cut to a contour drawing of the desired shape.

One of the major weaknesses of the prior art regarding three-dimensional porous scaffolds used for the regeneration of biological tissue like cartilage is that their microstructure is random. These scaffolds, unlike natural tissue, do not vary in morphology or structure. Further, current scaffolds do not provide adequate nutrient and fluid transport for many applications. Finally, the laminated structures are not completely integrated and subjected to delamination under in vivo conditions.

Therefore, it is an object of the present invention to provide a biocompatible, bioabsorbable foam that provides a continuous transitional gradient of morphological, structural and/or materials. Further, it is preferred that foams used in tissue engineering have a structure that provides organization at the microstructure level that provides a template that facilitates cellular invasion, proliferation and differentiation that will ultimately result in regeneration of functional tissue.

SUMMARY OF INVENTION

The present invention provides a biocompatible gradient foam that has a substantially continuous transition in at least one characteristic selected from the group consisting of composition, stiffness, flexibility, bioabsorption rate pore architecture and/or microstructure. This gradient foam can be made from a blend of absorbable polymers that form compositional gradient transitions from one polymeric material to a second polymeric material. In situations where a single chemical composition is sufficient for the application, the invention provides a biocompatible foam that may have microstructural variations in the structure across one or more dimensions that may mimic the anatomical features of the tissue (e.g. cartilage, skin, bone etc.).

The present invention further provides biocompatible foam having interconnecting pores and channels to facilitate the transport of nutrients and/or invasion of cells into the scaffold. These biocompatible foams are especially well adapted for facilitating the ingrowth of tissue as is described in Example 7.

In yet another embodiment of the present invention biocompatible foams having interconnecting pores formed from a composition containing in the range of from about 30

weight percent to about 99 weight ε-caprolactone repeating units are disclosed. These biocompatible foams are especially well adapted for facilitating the growth of osteoblasts as is described in Example 6.

The present invention also provides a method for the repair or regeneration of tissue contacting a first tissue with a gradient foam at a location on the foam that has appropriate properties to facilitate the growth of said tissue. The concept of a continuous transition in physical properties, chemical composition and/or microstructural features in the porous-scaffold (foam) can facilitate the growth or regeneration of tissue.

These foam structures are particularly useful for the generation of tissue junctions between two or more different types of tissues. For a multi-cellular system in the simplest case, one cell type could be present on one side of the scaffold and a second cell type on the other side of the scaffold. Examples of such regeneration can be (a) skin: with fibroblasts on one side to regenerate dermis, and keratinocytes on the other to regenerate epidermis; (b) vascular grafts: with an endothelial layer on the inside of the graft and a smooth muscle cell layer on the outside.

BRIEF DESCRIPTION OF FIGURES

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request;

FIG. **1** is a scanning electron micrograph of the cross section of a random microstructure foam made from 5% solution of 35/65 ε-caprolactone-co-glycolide copolymer.

FIG. **2** is a scanning electron micrograph of the cross section of a foam with vertical open channels made from 10% solution of 35/65 ε-caprolactone-co-glycolide copolymer.

FIG. 3 is a scanning electron micrograph of the cross section of a foam with architectural gradient made from 10% solution of 35/65 ε-caprolactone-co-glycolide copolymer.

FIG. **4** is a scanning electron micrograph of the cross section of a gradient foam made from a 50/50 blend of 40/60 ε-caprolactone-co-(L)lactide copolymer and 35/65 ε-caprolactone-co-glycolide copolymer.

FIG. **5** is a scanning electron micrograph of a cross section of the top portion of a gradient foam made from a 50/50 blend of 40/60 ε-caprolactone-co-(L)lactide copolymer and 35/65 ε-caprolactone-co-glycolide copolymer.

FIG. **6** is a scanning electron micrograph of a cross section of the bottom portion of a gradient foam made from a 50/50 blend of 40/60 ε-caprolactone-co-(L)lactide copolymer and 35/65 ε-caprolactone-co-glycolide copolymer.

FIG. 7 is a graphical presentation of cell culture data, 7A, 7B and 7C.

FIG. **8** is an anatomical sketch of cartilage tissue.

FIGS. 9A, 9B, and 9C are scanning electron micrographs of a 0.5 mm foam made from a 50/50 blend of a 35/65 ε-caprolactone-co-glycolide copolymer and a 40/60 ε-caprolactone-co-(L)lactide copolymer with architecture suitable for use as a skin scaffold. FIG. 9A shows the porosity of the surface of the scaffold that preferably would face the wound bed. FIG. 9B shows the porosity of the surface of the scaffolding that would preferably face away from the wound bed. FIG. 9C shows a cross section of the scaffold with channels running through the thickness of the foam.

FIG. **10** is a dark field 40× photomicrograph of a trichrome stained sample illustrating the cellular invasion of the foam shown in FIG. **9**, eight days after implantation in a swine model.

FIG. **11** is a 100× composite photomicrograph of a trichrome stained sample illustrating the cellular invasion of the foam shown in FIG. **9** which also contained PDGF, eight days after implantation in a swine model.

DETAILED DESCRIPTION OF THE INVENTION

This invention describes porous bioabsorbable polymer foams that have novel microstructures. The features of such foams can be controlled to suit a desired application by choosing the appropriate conditions to form the foam during lyophilization. These features in absorbable polymers have distinct advantages over the prior art where the scaffolds are typically isotropic or random structures. However, it is preferred that foams used in tissue engineering (i.e. repair or regeneration) have a structure that provides organization at the microstructural level that provides a template that facilitates cellular organization and regeneration of tissue that has the anatomical, biomechanical, and biochemical features of normal tissues. These foams can be used to repair or regenerate tissue (including organs) in animals such as domestic animals, primates and humans.

The features of such foams can be controlled to suit desired application by selecting the appropriate conditions for lyophilization to obtain one or more of the following properties: (1) interconnecting pores of sizes ranging from about 10 to about 200 μm (or greater) that provide pathways for cellular ingrowth and nutrient diffusion; (2) a variety of porosities ranging from about 20% to about 98% and preferably ranging from about 80% to about 95%; (3) gradient in the pore size across one direction for preferential cell culturing; (4) channels that run through the foam for improved cell invasion, vascularization and nutrient diffusion; (5) micro-patterning of pores on the surface for cellular organization; (6) tailorability of pore shape and/or orientation (e.g. substantially spherical, ellipsoidal, columnar); (7) anisotropic mechanical properties; (8) composite foams with a polymer composition gradient to elicit or take advantage of different cell response to different materials; (9) blends of different polymer compositions to create structures that have portions that will break down at different rates; (10) foams co-lyophilized or coated with pharmaceutically active compounds including but not limited to biological factors such as RGD's, growth factors (PDGF, TGF-β, VEGF, BMP, FGF etc.) and the like; (11) ability to make 3 dimensional shapes and devices with preferred microstructures; and (12) lyophilization with other parts or medical devices to provide a composite structure. These controlled features in absorbable polymers have distinct advantages over the prior art where the scaffolds are typically isotropic or random structures with no preferred morphology at the pore level. However, it is preferred that foams used in tissue scaffolds have a structure that provides organization at the microstructure level and provides a template that facilitates cellular organization that may mimic natural tissue. The cells will adhere, proliferate and differentiate along and through the contours of the structure. This will ultimately result in a cultured tissue that may mimic the anatomical features of real tissues to a large extent.

For example, as shown in FIG. **3** the orientation of the major axis of the pores may be changed from being in the same plane as the foam to being oriented perpendicular to the plane of the foam. As can be seen from FIG. 3 the pore size can be varied from a small pore size generally between

about 30 μm and about 50 μm to a larger size of from about 100 μm to about 200 μm in porous gradient foams. Ideally the foam structure could he created to facilitate the repair or regeneration of human tissuc junctions such as the cartilage to hone junction present in joints. This foam would progress from a small (i.e. about 30 μm to ahout 150 μm in diameter) round pores to larger column-like pores (i.e. ahout 30 μm to about 400 μm in diameter, preferahly about 100 μm to about 400 μm in diameter, in most cases with a length to diameter ratio of at least 2). Foams with channels are illustrated in FIG. 2 and FIG. 3. The channels formed hy this process generally begin on one surface of the foam and may traverse the thickness of the foam. The channel's length is generally at least two times the average pore diameter and preferably are at least four times the average pore diameter and most preferably at least eight times the average pore diameter. Channels for most applications will be at least 200 microns in length and may extend through the thickness of the foam. The diameter of the channel will be at least one time the size of thc average pore diameter and prcfcrably at least 2 to 3 times the average pore diameter. The channel size and diameter of course will he selected based on the desired functionality of the channel such as cellular invasion, nutrient diffusion or as an avenue for vascularization.

There are a numher of hiological tissues that demonstrate gradient architectures. Examples of tissues where a gradient scaffold could he used, include, hut are not limited to: hone, spine disc, articular cartilage, meniscus, fihrocartilage, tendons, ligaments, dura, skin, vascular grafts, nerves, liver, and pancreas. The examples helow only highlight a few tissues where gradient scaffolds could he used. The design of tissue enginccred scaffolds to facilitate development of these organ structures would benefit greatly from the ahility to process or create a gradient architecture in the scaffold.

Cartilage

Articular cartilage covers the ends of all hones that form articulating joints in humans and animals. The cartilage acts in the joint as a mechanism for force distrihution and as a bearing surface hetween different hones. Without articular cartilage, stress concentration and friction would occur to the degree that the joint would not permit ease of motion. Loss of the articular cartilage usually leads to painful arthritis and decreased joint motion. A schematic showing the morphological features of a healthy cartilage is shown in FIG. 8.

Articular cartilage is an excellent example of a naturally occurring gradient structure. Articular cartilage is composed of four different zones that include the superficial or tangential zone within the first 10–20% of the structure (this includes the articular surface), the middle zone which is 40–60% of the middle structure, and the deep zone that is adjaccnt to the tidc mark, and a transition zone hetween the hone and cartilage that is composed of calcified cartilage. Subchondral bone is located adjacent to the tide mark and this transitions into cancellous bone. In the superficial or tangential zone, the collagen fibrils are parallel to the surface. The fibers are oriented to resist shear forces generated during normal joint articulation. The middle zone has a randomly arranged organization of much larger diameter collagen fibers. Finally, in the deep zone there are larger collagen fiber bundles, which are perpendicular to the surface, and they insert into the calcified cartilage. The cells are speroidiol and tend to arrange themselves in a columnar manner. The calcified cartilage zone has smaller cells with relatively little cytoplasm.

A preferred embodiment of this invention would he to generate a gradient foam structure that could act as template for multiple distinct zones. These foam structures could be fabricated in a variety of shapes to regenerate or repair osteochondrial defects and cartilage. One potential foam structure would he cylindrical in shape with an approximate dimensions of 10 mm in diameter and 10 mm in depth. The top surface is would he approximately 1 mm thick and would he a low porosity layer to control the fluid permeahility. By adopting a suitahle processing method the surface porosity of the foam could he controlled. The porosity of this skin like surface can be varied from completcly impervious to completely porous. Fluid permeability would be controlled hy surface porosity. Below such a skin the structure would consist of three zones. An upper porous zone which lies adjacent to cartilage tissue, a lower porous zone which lies adjacent to bone tissue, and a transition zone between the upper and lower porous zones. For articular cartilage, it is currently preferred that the stiffness (modulus) of the upper and lower porous layers at the time of implantation be at least as stiff, as the corresponding adjacent tissue. In such a case the porous layers will be able to support the environmental loading and therehy protect the invading cells until they have differentiated and consolidated into tissue that is capahle of sustaining load. For example the porous structure used for the superficial tangential zone could have elongated pores and the orientation of the structure could be parallel to the surface of the host cartilage. However, the deep zone may have a porosity of ahout 80 to ahout 95 % with pores that are of the order of 100 μm (about 80 μm to about 120 μm). It is expected that chondrocytes will invade this zone. Below this, would hc a zone with larger pores (about 100 μm to ahout 200 μm) and a porosity in the range of about 50 to ahout 80%. Such 100 μm to about 200 μm porous foam would have a structure such that the struts or walls of the pores are larger and vertical to the load, similar to the naturally occurring structure and to bear the loads. Finally, at the hottom of this structure there is a need for larger pores (about 150 μm to ahout 300 μm) with higher stiffness to he structurally compatihle with cancellous hone. The foam in this section could he reinforced with ceramic particles or fibers made up of calcium phosphates and the like.

Recent data generated in our lahoratories support the hypothesis that cell invasion can be controlled by pore size. In these studies, a scaffold made of 95/5 mole percent poly(L) lactide-co-E-caprolactone) with an approximate pore size of about 80 μm had chondrocyte invasion of about 30 cells/mm^2 of the scaffold (under static conditions). Scaffolds made of 40/60 mole percent poly(ε-caprolactone-co-(L)lactide) with a larger approximate pore size of about 100 μm had a statistically significantly greater cellular invasion of 50 cells/mm^2 (under static conditions). In both cases the cells were bovine chondrocytcs. A very simple gradient structure with a variation of pore sized from ahout 80 μm to about 150 μm would provide a structure where chondrocytes would more easily invadc the area with largcr pores. The area with smaller pores would be void of chondrocytes or would he filled with a second cell types (e.g., fibroblasts).

In a compositionally gradient foam a blend of two or more elastomeric copolymers or in combination with high modulus semi-crystalline polymers along with additives such as growth factors or particulates can be chosen such that first a desired pore gradient is developed with a preferred spatial organization of the additives. Then using a variety of the approaches referred to in the preferred methods of making gradient foams, a compositional gradient can be superimposed primarily due to the differcnces in the polymcr-solvent phase separation hehavior of each system. Such a

gradient foam structure would elicit a favorable response to chondrocytes or osteoblasts depending on the spatial location.

Further, the purpose of a functional gradient is to more evenly distribute the stresses across a region through which mechanical and/or physical properties are varying and thereby alleviate the stress concentrating effects of a sudden interface. This more closely resembles the actual biological tissues and structures, where structural transitions between differing tissues such as cartilage and bone are gradual. Therefore, it is an object of the present invention to provide an implant with a functional gradient between material phases. The present invention provides a multi-phasic functionally graded bioabsorbable implant with attachment means for use in surgical repair of osteochondral defects or sites of osteoarthritis. Several patents have proposed systems for repairing cartilage that could be used with the present inventive porous scaffolds. For example, U.S. Pat. No. 5,769,899 describes a device for repairing cartilage defects and U.S. Pat. No. 5,713,374 describes securing cartilage repair devices with bone anchors (both hereby incorporated herein by reference).

Bone

Gradient structures naturally occur for the bone/cartilage interface. In a study in our laboratories, we have demonstrated that material differences significantly influence cell function. In initial and long-term response of primary osteoblasts to polymer films (95/5 L-lactide-co-glycolide copolymer, 90/10 glycolide-co-(L)lactide copolymer, 95/5 L-lactide-co-ε-caprolactone copolymer, 75/25 glycolide-co-(L)lactide copolymer and 40/60 ε-rolactone-co-(L)lactide copolymer and knitted meshes (95/5 (L)lactide-co-glycolide and 90/10 glycolide-co-(L)lactide copolymers) were evaluated in vitro. The results demonstrated that osteoblasts attached and proliferated well on all the biodegradable polymer films and meshes following 6-day incubation. None of the tested polymer films, except a 40/60 ε-caprolactone-co-(L)lactide copolymer film, demonstrated significant enhancement in differentiation of primary rat osteoblasts as compared to tissue culture polystyrene (control). Films made of 40/60-caprolactone-co-(L)lactide promoted enhanced differentiation of cultured osteoblasts as demonstrated by increased alkaline phosphatase activity and osteoclacin mRNA expression as compared to the other films and TCPS. Hence, it is clear that different absorbable materials will significantly alter cell function and differentiation. By identifying the optimal materials for cell growth and differentiation a composite materials with a gradient composition could be utilized to optimize tissue regeneration with different cell types in the same scaffold.

Therefore, for bone repair or regeneration devices or scaffoldings, a device made from a homopolymer, copolymer (random, block, segmented block, tappered blocks, graft, triblock, etc.) having a linear, branched or star structure containing ε-caprolactone is especially preferred. Currently preferred are aliphatic polyester copolymers containing in the range of from about 30 weight percent to about 99 weight percent ε-caprolactone. Suitable repeating units that may be copolymerized with ε-caprolactone are well known in the art. Suitable comonomers that may be copolymerized with ε-caprolactone include, but are not limited to lactic acid, lactide (including L-, D-, meso and D,L mixtures), glycolic acid, glycolide, p-dioxanone (1,4-dioxan-2-one), trimethylene carbonate (1,3-dioxan-2-one), δ-valerolactone, β-butyrolactone, ε-decalactone, 2,5-diketomorpholine, pivalolactone, α,α-diethylpropiolactone, ethylene carbonate, ethylene oxalate, 3-methyl-1,4-dioxane-2,5-dione, 3,3-diethyl-1,4-dioxan-2,5-dione, γ-butyrolactone, 1,4-dioxepan-2-one, 1,5-dioxepan-2-one, 6,6-dimethyldioxepan-2-one, 6,8-dioxabicycloctane-7-one and combinations thereof.

Preferred medical devices or tissue scaffoldings for bone tissue repair and/or regeneration containing bioabsorbable polymers made from ε-caprolactone include but are not limited to the porous foam scaffoldings (such as described in this application), fibrous three dimensional, spun, nonwoven, woven, knitted, or braided tissue scaffoldings, composite containing reinforcing fibers, matriees and combinations thereof.

Skin

Another example of a tissue that has a gradient structure is skin. The basic structure of skin has two distinct, but well integrated layers whcre the thickness of each layer varies at different locations of the body. The outer layer or epidermis, is avascular and mainly consists of keratinocytes with smaller numbers of immune cells (Langerhan cells) and pigmented cells (melanocytes). The keratinocytes produce keratin fibers and corneocyte envelopes, which gives the epidermis its durability and protective capabilities. The development of these structures is completely dependent upon the differentiation state of the epidermis. The epidermis forms a stratified epithelium, with different protein expression patterns, as the cells move further away from the basement membrane. This stratified layer of differentially expressing cells must be formed for maintenance of epidermal function. Below the epidermis is the dermis, which is a dense irregular connective tissue that is highly vascular. This layer is heavily populated with collageneic and elastic fibers, which give it its exceptional clasticity and strength. Fibroblasts are the main cell types in this layer. Between these two layers is the basement membrane, which serves as the site of attachment for epidermal cells and serves also to regulate their function and differentiation. The layer of keratinocytes, which attaches directly to the basement membrane, are cuboidal in shape and highly aligned. This attachment and architecture are critical requirements driving the ultimate production of the higher squamous structures in the epidermis. The basal layer provides a source of precursor cells for repair and replacement of the epidermis. The squamous layers provide strength and resistance to insult and infection.

Any material used for replacement of skin must be able to entice invasion of fibroblasts or other cells necessary to produce the dermal components of the healed tissue. Additionally, the material must not inhibit, and preferably should enhance, the rate of re-epithelialization in such a fashion that a discreet, epidermal basal layer is formed. Materials that permit invasion into the scaffold by migrating keratinocytes can produce partially differentiated cells. Consequently, control of access of particular cell types and a porous design that facilitates the regeneration of the natural tissue can have functional benefits. Now refer to FIGS. **9A, 9B** and **9C** which illustrates the microstructure of this foam scaffold. FIG. **10** (100×magnification) and 11 (40×magnification composite picture) provide photomicrographic evidence of the invasion of fibroblasts, macrophages, macrophage giant cells and endothelial-like cells into the a 0.5 mm foam. The foam tissue scaffolding **101** shown in both pictures was a 50:50 blend of ε-caprolactone-co-glycolide copolymer and ε-caprolactone-co-lactide copolymer (made as described in Example 7). The pictures were taken at 8 days after implantation in 1.5 cm×1.5 cm×0.2 cm exeisional wound model in a Yorkshire pig model. Complete incorporation of the matrix into the granulation tissue bed is evident in both pictures. The dense

fihrous tissue above tbe foam tissue scaffolding appears to provide a suitahle substrate for the over growth of epidermis. PDGF was incorporated into the foam tissue scaffolding shown in FIG. 11. In compromiscd wound healing models the addition of a growtb factor such as PDGF may in fact be necessary.

From our initial studies it appears that it is desirable to use as a skin scaffold a foam tissue scaffold baving a thickness of from ahout 150 μm to about 3 mm, preferably the thicknccss of the foam may be in the range of from about 300 μm to about 1500 μm and most preferahly about 500 to about 1000 μm. Clearly different skin injuries (i.e. diabetic ulcers, venous stasis ulcers, decubitis ulcers, burns etc.) may require different foam thickness. Additionally, the patient's condition may necessitate the incorporation of growth factors, antihiotics and antifungal compounds to facilitate wound healing.

Vascular Grafts

The creation of tubular structures with gradients may also be of interest. In vascular grafts, having a tube with pores in the outer diameter which transitions to smaller pores on the inner surface or visa versa may be useful in tbe culturing of endothelial cells and smooth muscle cells for the tissue culturing of vesscls.

Multilayered tuhular structures allow the regeneration of tissue that mimics the mechanical and/or hiological characteristics of hlood vessels will have utility as a vascular grafts. Concentric layers, made from different compositions under different processing conditions could have tailored mechanical properties, bioahsorption properties, and tissue ingrowth rates. The inner most, or luminal layer would be optimized for endothclialization through control of the porosity of the surface and the possible addition of a surface treatment. The outermost, or adventitial layer of the vascular graft would be tailored to induce tissue ingrowth, agaln hy optimizing the porosity (percent porosity, pore size, pore shape and pore size distribution) and by incorporating hioactive factors, pharmaceutical agents, or cells. There may or may not be a harrier layer with low porosity between these two porous layers to increase strength and decrease leakage.

Composition of Foams

A variety of absorbable polymers can be used to make foams. Examples of suitable hiocompatible, bioahsorhable polymers that could he used include polymers selected from the group consisting of aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylenes oxalates, polyamides, poly(iminocarhonates), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups, poly(anhydrides), polypbosphazenes, biomolecules and blends thereof. For the purpose of this invention aliphatic polycsters include hut are not limited to homopolymers and copolymers of lactide (whicb includes lactic acid, D-,L- and meso lactide), glycolide (including glycolic acid), ϵ-caprolactone, p-dioxanone (1,4-dioxan-2-one), trimethylene carbonate (1,3-dioxan-2-one), alkyl derivatives of trimetbylene carbonate, δ-valerolactone, β-hutyrolactone, γ-butyrolactone, ϵ-decalactone, hydroxybutyrate (repeating units), hydroxyvalerate (repeating units), 1,4-dioxepan-2-one (including its dimer 1,5,8,12-tetraoxacyclotetradecane-7,14-dione), 1,5-dioxepan-2-one, 6,6-dimethyl-1,4-dioxan-2-one 2,5-diketomorpboline, pivalolactone, alpha, alpha-diethylpropiolactone, ethylene carbonate, etbylene oxalate, 3-methyl-1,4-dioxane-2,5-dione, 3,3-diethyl-1,4-dioxan-2,5-dione, 6,8-dioxahicycloctane-7-onc and polymer blends thereof. Poly (iminocarbonate) for tbe purpose of this invention include as described by Kemnitzer and Kohn, in the *Handbook of Biodegradable Polymers*, edited by Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 251–272. Copoly(ether-esters) for the purpose of this invcntion include those copolyester-etbers described in "Journal of Biomaterials Research", Vol. 22, pages 993–1009, 1988 by Cohn and Younes and Cobn, Polymer Preprints (ACS Division of Polymer Chemistry) Vol. 30(1), page 498, 1989 (e.g. PEO/PLA). Polyalkylene oxalates for the purpose of tbis invention include U.S. Pat. Nos. 4,208,511; 4,141,087; 4,130,639; 4,140,678; 4,105,034; and 4,205,399 (incorporated by reference herein). Polyphospbazenes, co-, ter- and higher order mixed monomer based polymers made from L-lactide, D,L-lactide, lactic acid, glycolide, glycolic acid, para-dioxanone, trimetbylene carhonate and ϵ-caprolactone such as are described hy Allcock in *The Encyclopedia of Polymer Science*, Vol. 13, pages 31–41, Wiley Interscicnces, John Wiley & Sons, 1988 and by Vandorpe, Schacht, Dejardin and Lemmouchi in the *Handbook of Biodegradable Polymers*, edited hy Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 161–182 (which are hereby incorporated by reference herein). Polyanhydrides from diacids of the form HOOC—C_6H_4—O—$(CH_2)_m$—O—C_6H_4—COOH where m is an integer in the range of from 2 to 8 and copolymers thereof with aliphatic alpha-omega diacids of up to 12 carbons. Polyoxaesters, polyoxaamides and polyoxaesters containing amines and/or amido groups are described in one or more of the following U.S. Pat. Nos. 5,464,929; 5,595,751; 5,597,579; 5,607,687; 5,618,552; 5,620,698; 5,645,850; 5,648,088; 5,698,213; 5,700,583; and 5,859,150 (which are incorporated herein by reference). Polyorthoesters such as those described by Heller in *Handbook of Biodegradable Polymers*, edited by Domb, Kost and Wisemen, Hardwood Academic Press, 1997, pages 99–118 (hereby incorporated herein by reference).

Currently aliphatic polyesters are the ahsorbable polymers that are preferred for making gradient foams. Aliphatic polyesters can be homopolymers, copolymers (random, block, segmented, tappered blocks, graft, triblock, etc.) having a linear, hranched or star structure. Preferred are linear copolymers. Suitable monomers for making aliphatic homopolymers and copolymers may be selected from the group consisting of, hut are not limited, to lactic acid, lactide (including L-, D-, meso and D,L mixtures), glycolic acid, glycolide, ϵ-caprolactone, p-dioxanone (1,4-dioxan-2-one), trimethylene carbonate (1,3-dioxan-2-one), delta-valerolactone, beta-butyrolactone, epsilon-decalactone, 2,5-diketomorpholine, pivalolactone, alpha, alpha-diethylpropiolactone, ethylene carbonate, ethylene oxalate, 3-methyl-1,4-dioxane-2,5-dione, 3,3-diethyl-1,4-dioxan-2,5-dione, gamma-butyrolactone, 1,4-dioxcpan-2-onc, 1,5-dioxepan-2-one, 6,6-dimethyl-dioxepan-2-one, 6,8-dioxabicycloctane-7-one and comhinations thereof.

Elastomeric copolymers also are particularly useful in the present invention. Suitable bioabsorbable hiocompatible elastomers include but are not limited to those selected from the group consisting of elastomeric copolymers of ϵ-caprolactone and glycolide (preferably having a mole ratio of ϵ-caprolactone to glycolide of from ahout 35:65 to ahout 65:35, more preferably from 45:55 to 35:65) elastomeric copolymers of ϵ-caprolactone and lactide, including L-lactide, D-lactide hlends thereof or lactic acid copolymers (preferably having a mole ratio of ϵ-caprolactone to lactide of from about 35:65 to about 65:35 and more preferably from 45:55 to 30:70 or from about 95:5 to ahout 85:15) elastomeric copolymers of p-dioxanone (1,4-dioxan-2-one)

and lactide including L-lactide, D-lactide and lactic acid (preferably having a mole ratio of p-dioxanone to lactide of from about 40:60 to about 60:40) elastomeric copolymers of ε-caprolactone and p-dioxanone (preferably having a mole ratio of ε-caprolactone to p-dioxanone of from about from 30:70 to about 70:30) elastomeric copolymers of p-dioxanone and trimethylene carbonate (preferably having a mole ratio of p-dioxanone to trimethylene carbonate of from about 30:70 to about 70:30), elastomeric copolymers of trimethylene carbonate and glycolide (preferably having a mole ratio of trimethylene carbonate to glycolide of from about 30:70 to about 70:30), elastomeric copolymer of trimethylene carbonate and lactide including L-lactide, D-lactide, blends thereof or lactic acid copolymers (preferably having a mole ratio of trimethylene carbonate to lactide of from about 30:70 to about 70:30) and blends thereof. Examples of suitable bioabsorbable elastomers are described in U.S. Pat. Nos. 4,045,418; 4,057,537 and 5,468, 253 all hereby incorporated by reference. These elastomeric polymers will have an inherent viscosity of from about 1.2 dL/g to about 4 dL/g, preferably an inherent viscosity of from about 1.2 dL/g to about 2 dL/g and most preferably an inherent viscosity of from about 1.4 dL/g to about 2 dL/g as determined at 25° C. in a 0.1 gram per deciliter (g/dL) solution of polymer in hexafluoroisopropanol (HFIP).

Preferably, the elastomers will exhibit a high percent elongation and a low modulus, while possessing good tensile strength and good recovery characteristics. In the preferred embodiments of this invention, the elastomer from which the foams are formed will exhibit a percent elongation greater than about 200 percent and preferably greater than about 500 percent. There properties, which measure the degree of elasticity of the bioabsorbable elastomer, are achieved while maintaining a tensile strength greater than about 500 psi, preferably greater than about 1,000 psi, and a tear strength of greater than about 50 lbs/inch, preferably greater than about 80 lbs/inch.

The polymer or copolymer suitable for forming a gradient foam for tissue regeneration depends on several factors. The chemical composition, spatial distribution of the constituents, the molecular weight of the polymer and the degree of crystallinity all dictate to some extent the in-vitro and in-vivo behavior of the polymer. However, the selection of the polymers to make gradient foams for tissue regeneration largely depends on (but not limited to) the following factors: (a) bio-absorption (or bio-degradation) kinetics; (b) in-vivo mechanical performance; and (c) cell response to the material in terms of cell attachment, proliferation, migration and differentiation and (d) biocompatibility.

The ability of the material substrate to resorb in a timely fashion in the body environment is critical. But the differences in the absorption time under in-vivo conditions can also be the basis for combining two different copolymers. For example a copolymer of 35:65 ε-caprolactone and glycolide (a relatively fast absorbing polymer) is blended with 40:60 ε-caprolactone and (L)lactide copolymer (a relatively slow absorbing polymer) to form a foam. Such a foam could have several different physical structures depending upon the processing technique used. The two constituents can be either randomly inter-connected bicontinuous phases, or the constituents can have a gradient through the thickness or a laminate type composite with a well integrated interface between the two constituent layers. The microstructure of these foams can be optimized to regenerate or repair the desired anatomical features of the tissue that is being engineered.

One preferred embodiment of the present invention is to use polymer blends to form structures which transition from one composition to another composition in a gradient like architecture. Foams having this gradient architecture are particularly advantageous in tissue engineering applications to repair or regenerate the structure of naturally occurring tissue such as cartilage (articular, meniscal, septal, tracheal etc.), esophaguses, skin, bone and vascular tissue. For example by blending an elastomer of ε-caprolactone-co-glycolide with ε-caprolactone-co-lactide (i.e. with a mole ratio of about 5:95) a foam may be formed that transitions from a softer spongy foam to a stiffer more rigid foam similar to the transition from cartilage to bone. Clearly other polymer blends may be used for similar gradient effects or to provide different gradients such as different absorption profiles, stress response profiles, or different degrees of elasticity. Additionally, these foams can be used for organ repair replacement or regeneration strategies that may benefit from these unique scaffolds, including but are not limited to, spine disc, dura, nerve tissue, liver, pancreas, kidney, bladder, tendons, ligaments and breast tissues.

These elastomeric polymers may be foamed by lyophilization, supercritical solvent foaming (i.e., as described in EP 464,163 B1), gas injection extrusion, gas injection molding or casting with an extractable material (i.e., salts, sugar or any other means known to those skilled in the art). Currently it is preferred to prepare bioabsorbable, biocompatible elastomeric foams by lyophilization. Suitable methods for lyophilizing elastomeric polymers to form foams is described in the Examples and in the copending patent application entitled, "Process for Manufacturing Biomedical Foams", assigned to Ethicon, Inc., Ser. No. 09/345, 095, filed Jun. 30, 1999 hereby incorporated herein by reference herein.

The foams that are made in this invention are made by a polymer-solvent phase separation technique with modifications to the prior art that unexpectedly creates gradients in the foam structure. Generally, a polymer solution can be separated into two phases by any one of the four techniques: (a) thermally induced gelation/crystalization; (b) non-solvent induced separation of solvent and polymer phases; (c) chemically induced phase separation, and (d) thermally induced spinodal decomposition. The polymer solution is separated in a controlled manner into either two distinct phases or two bicontinuous phases. Subsequent removal of the solvent phase usually leaves a porous structure of density less than the bulk polymer and pores in the micrometer ranges (ref. "Microcellular foams via phase separation" by A. T. Young, J. Vac. Sci. Technolol. A 4(3), May/June 1986). The steps involved in the preparation of these foams consists of choosing the right solvents for the polymers that needs to be lyophilized and preparing a homogeneous solution. Next, the polymer solution is subjected to a freezing and vacuum drying cycle. The freezing step phase separates the polymer solution and vacuum drying step removes the solvent by sublimation and/or drying leaving a porous polymer structure or an interconnected open cell porous foam.

Suitable solvents that should be generally suited as a starting place for selecting a solvent for the preferred absorbable aliphatic polyesters include but are not limited to solvents selected from a group consisting of formic acid, ethyl formate, acetic acid, hexafluoroisopropanol (HFIP), cyclic ethers (i.e. THF, DMF, and PDO), acetone, acetates of C2 to C5 alcohol (such as ethyl acetate and t-butylacetate), glyme (i.e. monoglyme, ethyl glyme, diglyme, ethyl diglyme, triglyme, butyl diglyme and tetraglyme) methyl-ethyl ketone, dipropyleneglycol methyl ether, lactones (such as γ-valerolactone, δ-valerolactone, β-butyrolactone, γ-butyrolactone) 1,4-dioxane, 1,3-dioxolane, 1,3-dioxolane-

2-one (ethylene carbonate), dimethlycarbonate, benzene, toluene, benzyl alcohol, p-xylene, naphthalene, tetrahydrofuran, N-methyl pyrrolidone, dimethylformamide, chloroform, 1,2-dichloromethane, morpholine, dimethylsulfoxide, hexafluoroacetone sesquihydrate (HFAS), anisole and mixtures thereof. Among these solvents, the preferred solvent is 1,4-dioxane. A homogeneous solution of the polymer in the solvent is prepared using standard techniques.

Accordingly, as will be appreciated, the applicable polymer concentration or amount of solvent, which may be utilized, will vary with each system. Suitable phase diagram curves for several systems have already been developed. However, if an appropriate curve is not available, this can be readily developed by known techniques. For example, a suitable technique is set forth in Smolders, van Aartsen and Steenbergen, Kolloid-Z. u. Z. Polymere, 243, 14 (1971). As a general guideline the amount of polymer in the solution can vary from about 0.5% to about 90% and preferably will vary from about 0.5% to about 30% by weight depending to a large extent on the solubility-of-the polymer in a given solvent and the final properties of the foam desired.

Additionally, solids may be added to the polymer-solvent system. The solids added to the polymer-solvent system preferably will not react with the polymer or the solvent. Suitable solids include materials that promote tissue regeneration or regrowth, buffers, reinforcing materials or porosity modifiers. Suitable solids include, but are not limited to, particles of demineralized bone, calcium phosphate particles, or calcium carbonate particles for bone repair, leachable solids for pore creation and particles of bioabsorbable polymers not soluble in the solvent system as reinforcing or to create pores as they are absorbed. Suitable leachable solids include but are not limited nontoxic leachable materials selected from the group consisting of salts (i.e. sodium chloride, potassium chloride, calcium chloride, sodium tartrate, sodium citrate, and the like) biocompatible mono and disaccharides (i.e. glucose, fructose, dextrose, maltose, lactose and sucrose), polysaccharides (i.e. starch, alginate), water soluble proteins (i.e. gelatin and agarose). Generally all of these materials will have an average diameter of less than about 1 mm and preferably will have an average diameter of from about 50 to about 500 μm. The particles will generally constitute from about 1 to about 50 volume percent of the total volume of the particle and polymer-solvent mixture (wherein the total volume percent equals 100 volume percent). The leachable materials can be removed by immersing the foam with the leachable material in a solvent in which the particle is soluble for a sufficient amount of time to allow leaching of substantially all of the particles, but which does not dissolve or detrimentally alter the foam. The preferred extraction solvent is water, most preferably distilled-deionized water. This process is described in U.S. Pat. No. 5,514,378 hereby incorporated herein by reference (see column 6). Preferably the foam will be dried after the leaching process is complete at low temperature and/or vacuum to minimize hydrolysis of the foam unless accelerated absorption of the foam is desired.

After the polymer solvent mixture is formed the mixture is then solidified. For a specific polymer-solvent system, the solidification point, the melt temperature and the apparent glass transition of the polymer-solvent system can be determined using standard differential scanning calorimetric (DSC) techniques. In theory, but in no way limiting the scope of the present invention, it is believed that as a polymer solvent system is cooled down an initial solidification occurs at about or below the freezing point of the solvent. This corresponds to the freezing of a substantial portion of the solvent in the system. The initial freezing appears as a first exothermic peak. A second freezing point occurs when the remaining solvent associated with the polymer solidifies. The second freezing point is marked by a second exothermic peak. The apparent Tg is the temperature at which the fully frozen system displays the first endothermic shift on reheating.

An important parameter to control is the rate of freezing of the polymer-solvent system. The type of pore morphology that gets locked in during the freezing step is a function of the solution thermodynamics, freezing rate, temperature to which it is cooled, concentration of the solution, homogeneous or heterogenous nucleation etc. Detailed description of these phase separation phenomenon can be found in the references provided herein ("Microcellular foams via phase separation" by A. T. Young, J. Vac. Sci. Technol. A 4(3), May/June 1986; and "Thermodynamics of Formation of Porous Poymeric Membrane from Solutions" by S. Matsuda, Polymer J. Vol. 23, No. 5, pp 435–444, 1991).

The polymer solution previously described can be solidified in a variety of manners stch as placing or injecting the solution in a mold and cooling the mold in an appropriate bath or on a refrigerated shelf. Alternatively, the polymer solution can be atomized by an atomizer and sprayed onto a cold surface causing solidification of the spray layer by layer. The cold surface can be a medical device or part thereof or a film. The shape of the solidified spray will be similar to the shape of the surface it is sprayed onto. Alternatively, the mixture after solidification can be cut or formed to shape while frozen. Using these and other processes the foams can be made or molded in a variety of shapes and sizes (i.e. tubular shapes, branched tubular shapes, spherical shapes, hemispherical shapes, three-dimensional polygonal shapes, ellipsoidal shapes (i.e. kidney shaped), toroidal shapes, conical shapes, frusta conical shapes, pyramidal shapes, both as solid and hollow constructs and combination thereof).

Alternatively, another method to make shaped foamed parts is to use a cold finger (a metal part whose surface represents the inside of the part we want to fabricate). The cold finger is dipped into a mixture of polymer in an appropriate solvent and removed. This is much like dipping an ice cream pop into warm chocolate that freezes to a hard, cold skin, or dipping a form into a latex of rubber to form gloves or condoms. The thickness and morphology of the foam produced are a function of the temperature, dwell time and withdrawal rate of the cold finger in the mixture. Longer dwell, colder finger and slower withdrawal will produce a thicker coating. After withdrawal, the cold finger is placed on a fixture of large thermal mass that is in contact with the refrigerated tray of the lyophilizer. From this point the primary and secondary drying processes are as described above. This method is particularly well suited to making tubes, branched tubular structures or sleeves that may be shaped to fit devices or portions of an animal's anatomy (for repair, regeneration or augmentation of tissue).

Additionally, the polymer solution can be solidified with various inserts incorporated with the solution such as films, scrims, woven, nonwoven, knitted or braided textile structures. Additionally, the solution can be prepared in association with another structure such an orthopedic implant (e.g. screws, pins, nails, and plates) or vascular or branched tubular construct (as a scaffold for a vascularized or ducted organ). These inserts will be made of at least one biocompatible material and may be non-absorbable, absorbable or a combination thereof.

The polymer solution in a mold undergoes directional cooling through the wall of the mold that is in contact with the freeze dryer shelf, which is subjected to a thermal cycle. The mold and its surface can be made from virtually any material that does not interfere with the polymer-solvent system, though it is preferred to have a highly conducting material. The heat transfer front moves upwards from the lyophilizer shelf through the mold wall into the polymer solution. The instant the temperature of the mixture goes below the gellation and/or freezing point the mixture also phase separates.

The morphology of this phase separated system is locked in place during the freezing step of the lyophilization process and the creation of the open pores is initiated by the onset of vacuum drying resulting in the sublimation of the solvent. However, the mixture in container or mold that is cooled from a heat sink will solidify prior to completely freezing. Although the mixture may appear solid, initially there appears to be some residual solvent associated with the polymer that has not cystallized. It is theorized, but in no way limiting the present invention, that a freezing front moves through the mixture from the heat sink to complete the solidification after the mixture has apparently solidified. The material in front of the freezing front at a given time will not be as cold as the material behind the front and will not be in a completely frozen state.

We have discovered that if a vacuum is applied to the apparently solid polymer-solvent mixture immediately after it appears to solidify, a foam with a gradient structure having variable pore size and structure as well as channels can be created. Therefore, timing of the onset of the sublimation process (by pressure reduction i.e. vacuum drying) is a critical step in the process to create transitions in the structure. The timing of the onset of sublimation will be affected by the thickness of the foam being made, concentration of the solution, rate of heat transfer, and directionalities of the heat transfer. Those skilled in the art will appreciate that this process can be monitored and characterized for specific polymer-solvent systems by using thermocouples and monitoring the heat transfer rates of the foams at various depths and locations in the device being foamed. By controlling the sublimation process, structures with a gradient in pore morphology and anisotropy may be created. This process can lead to the creation of microstructures that mimic tissues such as cartilage, bone and skin. For example, channels will generally be formed if a vacuum is pulled immediately after the solution apparently solidifies. However, if the same solution is allowed to solidify further the foam will have larger pores closer to the surface where the vacuum is being drawn (opposite the heat sink) and smaller pores closer to the heat sink.

This process is the antitheses of the prior art process that focused on creating foams with a uniform microstructure (randomly interconnected pores), whereby whole solution is completely frozen. And vacuum drying is applied only after a considerable amount of time is given for the completion of desired phase separation (U.S. Pat. Nos. 5,755,792 (Brekke); 5,133,755 (Brekke); 5,716,413 (Walter, et al.); 5,607,474 (Athanasiou, et al.); 5,686,091 (Leong, et al.); 5,677,355 (Shalaby, et al.); and European disclosures E0274898 (Hinsch) and EPA 594148 (Totakura)).

Foams with various structures are shown in FIGS. 2, 3, and 4. For example, as shown in FIG. 3 the orientation of the major axis of the pores may be changed from being in the same plane as the foam to being oriented perpendicular to the plane of the foam. By way of theory, but in no way limiting the scope of this invention, it is believed that this in conventional foam processing as the solvent crystallizes a freezing front moves through the solution solidifying the solution in crystalline layers parallel to the freezing front. However, if a vacuum is pulled before the solution completely freezes, the morphology of the foam results in pores being formed generally aligned parallel to the vacuum source. As is illustrated in FIG. 3.

As can be seen from FIG. 3 the pore size can be varied from a small pore size generally between about 10 μm and about 60 μm to a larger size of from about 60 μm to about 200 μm in a porous gradient foam. Again this results from pulling a vacuum on the apparently solidified solution before it is completely solidified. The polymer concentration in the solution and the cooling rates are also important parameters in controlling the cell size. Ideally the foam structure could be created to serve as a template to restore human tissue junctions such as the cartilage to bone junction present in joints. This foam would progress form a small round pores to larger column-like (i.e. with a diameter to length ratio of at least 2 to 1) pores. Additionally, the stiffness of the foam can controlled by the foams structure or blending two different polymers with different Young's moduli.

Foams can also have channels as is illustrated in FIG. 2. The channels formed by this process may traverse the thickness of the foam and generally range in diameter from about 30 to about 200 μm in diameter. The channels generally are at least two times the channel's average diameter and preferably are at least four times the channel's average diameter and most preferably at least eight times the channel's average diameter. The channel size and diameter of course will be selected based on the desired functionality of the channel such as cell invasion, nutrient diffusion or as a avenue for vascularization.

One skilled in the art can easily visualize that such a directionality can be created in any three dimensions by designing an appropriate mold and subjecting the walls of such a mold to different temperatures if needed. The following types of gradient structures can be made by variation in the pore size and/or shape through the thickness with a uniform composition: pores of one type and size for a certain thickness followed by another type and size of pores, etc; compositional gradient with predominantly one composition on one side and another one on the other with a transition from one entity to the other; a thick skin comprising low porosity of low pore size layer followed by a large pore size region; foams with vertical pores with a spatial organization these vertical pores can act as channels for nutrient diffusion the making of these in 3D molds to create 3D foams with the desired microstructure combinations of compositional and architectural gradient. Generally the foams formed in containers or molds will have a thickness in the range of from about 0.25 mm to about 100 mm and preferably will have a thickness of from about 0.5 mm to about 50 mm. Thicker foams can be made but the lyophilization cycle times may be quite long, the final foam structures may be more difficult to control and the residual solvent content may be higher.

As previously described the inventive process cycle for producing biocompatible foam is significantly reduced by performing the sublimation step above the apparent glass transition temperature and below the solidification temperature of the mixture (preferably just below the solidification temperature). The combined cycle time of (freezing+primary drying+secondary drying) is much faster than is described in the prior art. For example, the combined cycle for aliphatic polyesters using volatile solvents is generally less than 72 hours, preferably less than 48 hours, more preferably less than 24 hours and most preferably less than

10 hours. In fact the combined cycle can be performed with some aliphatic polyesters in less than 3 hrs for foams of thickness 1 mm or less; less than 6 hrs for foams of thickness around 2 mm and less than 9 hrs for foams of thickness around 3 mm. Compare this with prior art which is typically 72 hours or greater. The residual solvent concentrations in these foams made by this process will be very low. As described for aliphatic polyesters foams made using 1,4-dioxane as a solvent the residual concentration of 1,4-dioxane was less than 10 ppm (parts per million) more preferably less than 1 ppm and most preferably less than 100 ppb (parts per billion).

One skilled in the art can easily visualize that such a directionality can be created in any three-dimensions by designing an appropriate mold and subjecting the walls of such a mold to different temperatures if needed. The following types of gradient structures can be made by this invention.

1. variation in the pore size and/or shape through the thickness with a uniform composition,
2. pores of one type and size for a certain thickness followed by another type and size of pores, etc
3. compositional gradient with predominantly one compostion on one side and another composition on the other with a transition from one entity to the other
4. a thick skin comprising low porosity of low pore size layer followed by a large pore size region
5. foams with vertical pores with a spatial organization ... these vertical pores can act as channels for nutrient diffusion
6. the making of these in three-dimensional molds to create three-dimensional foams with the desired microstructure.
7. combinations of compositional and architectural gradient

Additionally, various proteins (including short chain peptides), growth agents, chemotatic agents and therapeutic agents (antibiotics, analgesics, anti-inflammatories, anti-rejection (e.g. immunosuppressants) and anticancer drugs), or ceramic particles can be added to the foams during processing, adsorbed onto the surface or back filled into the foams after the foams are made. For example, the pores of the foam may be partially or completely filled with biocompatible resorbable synthetic polymers or biopolymers (such as collagen or elastin) or biocompatible ceramic materials (such as hydroxyapatite) and combinations thereof (that may or may not contain materials that promote tissue growth through the device). Suitable materials include but are not limited to autograft, allograft, or xenograft bone, bone marrow, morphogenic proteins (BMP's), epidermal growth factor (EGF), fibroblast growth factor (FgF), platelet derived growth factor (PDGF), insulin derived growth factor (IGF-I and IGF-II), transforming growth factors (TGF-P), vascular endothclial growth factor (VEGF) or other osteoinductive or osteoconductive materials known in the art. Biopolymers could also be used as conductive or chemotactic materials, or as delivery vehicles for growth factors. Examples could be recombinant or animal derived collagen or elastin or hyaluronic acid. Bioactive coatings or surface treatments could also be attached to the surface of the materials. For example, bioactive peptide sequences (RGD's) could be attached to facilitate protein adsorption and subsequent cell tissue attachment. Therapeutic agents may also be delivered with these foams.

In another embodiment of the present invention, the polymers and blends that are used to form the foam can contain therapeutic agents. To form these foams, the previously described polymer would be mixed with a therapeutic agent prior to forming the foam or loaded into the foam after it is formcd. The variety of different therapeutic agents that can be used in conjunction with the foams of the present invention is vast. In general, therapeutic agents which may be administered via the pharmaceutical compositions of the invention include, without limitation: antiinfeetives such as antibiotics and antiviral agents; chemotherapeutic agents (i.e. anticancer agents); anti-rejection agents; analgesics and analgesic combinations; anti-inflammatory agents; hormones such as steroids; growth factors (bone morphogenic proteins (i.e. BMP's 1–7), bone morphogenic-like proteins (i.e. GFD-5, GFD-7 and GFD-8), epidermal growth factor (EGF), fibroblast growth factor (i.e. FGF 1–9), platelet derived growth factor (PDGF), insulin like growth factor (IGF-I and IGF-II), transforming growth factors (i.e. TGF-P I-III), vascular cndothelial growth factor (VEGF)); and other naturally derived or genetically engineered proteins, polysaccharides, glycoproteins, or lipoproteins. These growth factors are described in *The Cellular and Molecular Basis of Bone Formation and Repair* by Vicki Rosen and R. Scott Thies, published by R. G. Landes Company hereby incorporated herein by reference.

Foams containing bio-active materials may be formulated by mixing one or more therapeutic agents with the polymer used to make the foam or with the solvent or with the polymer-solvent mixture and foamed. Alternatively, a therapeutic agent could be coated on to the foam preferably with a pharmaceutically acceptable carrier.

Any pharmaceutical carrier can be used that does not dissolve the foam. The therapeutic agents, may be present as a liquid, a finely divided solid, or any other appropriate physical form. Typically, but optionally, the matrix will include one or more additives, such as diluents, carriers, excipients, stabilizers or the like.

The amount of therapeutic agent will depend on the particular drug being employed and medical condition being treated. Typically, the amount of drug represents about 0.001 percent to about 70 percent, more typically about 0.001 percent to about 50 percent, most typically about 0.001 percent to about 20 percent by weight of the matrix. The quantity and type of polymer incorporated into the drug delivery matrix will vary depending on the release profile desired and the amount of drug employed.

Upon contact with body fluids the drug will be released. If the drug is incorporated into the foam then as the foam undergoes gradual degradation (mainly through hydrolysis) the drug will be released. This can result in prolonged delivery (over, say 1 to 5,000 hours, preferably 2 to 800 hours) of effective amounts (say, 0.0001 mg/kg/hour to 10 mg/kg/hour) of the drug. This dosage form can be administered as is necessary depending on the subject being treated, the severity of the affliction, the judgment of the prescribing physician, and the like. Following this or similar procedures, those skilled in the art will be able to prepare a variety of formulations.

The foam may also serve as a scaffold for the engineering of tissue. The porous gradient structure would be conducive to growth of cells. As outlined in previous patents (Vacanti, U.S. Pat. No. 5,770,417), cells can be harvested from a patient (before or during surgery to repair the tissue) and the cells can be processed under sterile conditions to provide a specific cell type (i.e., pluripotent cells, stem cells or precursor cells such as the mesenchymal stem cells described in Caplan, U.S. Pat. No. 5,486,359, etc.). Suitable cell that may be contacted or seeded into the foam scaffolds include but

are not limited to myocytes, adipocytes, fibromyoblasts, ectodermal cell, muscle cells, osteoblast (i.e. bone cells), chondrocyte (i.e. cartilage cells), endothelial cells, fibroblast, pancreatic cells, hepatocyte, bile duct cells, bone marrow cells, neural cells, genitourinary cells (including nephritic cells) and combinations thereof. Various cellular strategies could be used with these scaffolds (i.e., autogenous, allogenic, xenogeneic cells etc.). The cells could also contain inserted DNA encoding a protein that could stimulate the attachmnent, proliferation or differentiation of tissue. The foam would be placed in cell culture and the cells seeded onto or into the structure. The foam would be maintained in a sterile environment and then implanted into the donor patient once the cells have invaded the microstructure of the device. The in vitro seeding of cells could provide for a more rapid development and differentiation process for the tissue. It is clear that cellular differentiation and the creation of tissue specific extracellular matrix is critical for the tissue engineering of a functional implant.

The option for seeding different cell types into the different pore structures would be available to investigators. Schaufer et al., have demonstrated that different cell types (i.e. stromal cells and chondrocytes) can be cultured on different structures. The structures can bc combined after a short period of time and the entire structure can be placed back in cell culture so a biphasic tissue structure can be generated for implantation. A gradient structure would also allow for co-cultured tissue scaffolds to be generated. (Schaefer, D. et al.). Additionally, radio-opaque markers may be added to the foams to allow imaging after implantation. After a defined period of in vitro development (for example 3 weeks), the tissue engincered implant would be harvested and implanted into the patient. If an a cellular strategy is pursued, then the sterile acellular scaffolds would be used to replace damaged or traumatized tissue.

The foam scaffolds of the present invention may be sterilized using conventional sterilization process such as radiation based sterilization (i.c. gamma-ray), chemical based sterilization (ethylene oxide) or other appropriate procedures. Preferably the sterilization process will be with ethylene oxide at a temperature between 52–55° C. for a time of 8 hours or less. After sterilization the foam scaffolds may be packaged in an appropriate sterilize moisture resistant package for shipment and use in hospitals and other health care facilities.

Thc following examplcs are illustrative of the principles and practice of this invention, although not limited thereto. Numerous additional embodiments within the scope and spirit of the invention will become apparent to those skilled in the art.

EXAMPLES

In the examples which follow, the polymers and monomers were characterized for chemical composition and purity (NMR, FT-IR), thermal analysis (DSC), molecular weight (inherent viscosity), and baseline and in vitro mechanical properties (Instron stress/strain).

^{1}H NMR was performed on a 300 MHz NMR using $CDCl_3$ or HFAD (hexafluoroacetone sesqua deutrium oxide) as a solvent. Thermal analysis of segmented polymers and monomers was performed on a Dupont 912 Differential Scanning Calorimeter (DSC). Inherent viscosities (I.V., dL/g) of the polymers and copolymers were measured using a 50 bore Cannon-Ubbelhode dilution viscometer immersed in a thermostatically controlled water bath at 25° C. utilizing chloroform or hexafluoroisopropanol (HFIP) as the solvent at a concentration of 0.1 g/dL.

In these examples certain abbreviations are usde such as PCL to indicate polymerized ϵ-caprolactone, PGA to indicate polymerized glycolide, PLA to indicate polymerized (L)lactide. Additionally, the percentages in front of the copolymer indicates the respective mole percentages of each constituent.

Example 1

Preparation of a Foam with Random Microstructure (No Preferred Architecture)

Step A. Preparing 5% wt./wt. homogeneous solution of 35/65 PCL/PGA in 1,4-Dioxane

A 5% wt./wt. polymer solution is prepared by dissolving 1 part of 35/65 PCL/PGA with 19 parts of the solvent-1,4-dioxane. The 35/65 PCL/PGA copolymer was made substantially as described in Example 8. Thc solution is prepared in a flask with a magnetic stir bar. For the copolymer to dissolve completely, it is recommended that the mixture is gently heated to 60±5° C. and continuously stirred for a minimum of 4 hours but not exceeding 8 hours. A clear homogeneous solution is then obtained by filtering the solution through an extra coarse porosity filter (Pyrex brand extraction thimble with fritted disc) using dry nitrogen to help in the filtration of this viscous solution.

Step B. Lyophilization

A laboratory scale lyophilizer—Freezemobile 6 of VIRTIS was used in this experiment. The freeze dryer is powered up and the shelf chamber is maintained at 20° C. under dry nitrogen for approximately 30 minutes. Thermocouples to monitor the shelf temperature are attachcd for monitoring. Carefully fill the homogeneous polymer solution prepared in Step A. into the molds just before the actual start of the cycle. A glass mold was used in this example but a mold made of any material that is inert to 1,4-dioxane; has good heat transfer characteristics; and has a surface that enables the easy removal of the foam can be used. The glass mold or dish used in this example weighed 620 grams, was optical glass 5.5 mm thick, and cylindrical with a 21 cm outer diameter and a 19.5 cm inner diameter. The lip height of the dish was 2.5 cm. Next the following steps are followed in a sequence to make a 2 mm thick foam:

(i). The glass dish with the solution is carefully placed (without tilting) on the shelf of the lyophilizer, which is maintained at 20° C. The cycle is started and the shelf temperature is held at 20° C. for 30 minutes for thermal conditioning.

(ii). The solution is then cooled to −5° C. by cooling the shelf to −5° C.

(iii). After 60 minutes of freezing at −5° C., a vacuum is applied to initiate primary drying of the dioxane by sublimation. One hour of primary drying under vacuum at −5° C. is needed to remove most of the solvent. At the end of this drying stage typically the vacuum level reached about 50 mTorr or less.

(iv). Next, secondary drying under a 50 mTorr vacuum or less was done in two stages to remove the adsorbed dioxane. In the first stage, the shelf temperature was raised to 5° C. and held at that temperature for 1 hour. At the end of the first stage the second stage of drying was begun. In the second stage of drying, the shelf temperature was raised to 20° C. and held at that temperature for 1 hour.

(v). At the end of the second stage, the lyophilizer is brought to room temperature and the vacuum is broken with nitrogen. The chamber is purged with dry nitrogen for approximately 30 minutes before opening the door.

The steps described above are suitable for making foams that are about 2 mm thick or less. As one skilled in the art

would know, the conditions described herein are typical and operating ranges depend on several factors e.g.: concentration of the solution; polymer molecular weights and compositions; volume of the solution; mold parameters; machine variables like cooling rate, heating rates; and the like. FIG. 1 shows a SEM of a cross section of the foam produced following the process set forth in this example. Note the random microstructure (not a preferred architecture) of this foam.

Example 2

Preparation of a Foam with Vertical Channels

This example describes the making of a 35/65 PCL/PGA foam with vertical channels that would provide pathways for nutrient transport and guided tissue regeneration.

We used a FTS Dura Dry Freeze dryer with computer control and data monitoring system to make this foam. First step in the preparation of this foam was to generate a homogeneous solution. A 10% wt./wt. homogeneous solution of 35/65 PCL/PGA was made in a manner similar to that described in Example 1, Step A. The polymer solution was carefully filled into a dish just before tbe actual start of the cycle. The dish weighed 620 grams, was optical glass 5.5 mm thick, and cylindrical with a 21 cm outer diameter and a 19.5 cm inner diameter. The lip height of the dish was 2.5 cm. Next the following steps are followed in sequence to make a 2 mm thick foam with the desired architecture:

(i). The solution filled dish was placed on the freeze dryer shelf that was precooled to −17° C. The cycle was started and the shelf temperature was held at −17° C. for minutes quenching the polymer solution.

(ii). After 15 minutes of quenching to −17° C., a vacuum was applied to initiate primary drying of the dioxane by sublimation and held at 100 milliTorr for one hour.

(iii). Next, secondary drying was done at 5° C. for one hour and at 20° C. for one hour. At each temperature the vacuum level was maintained at 20 mTorr.

(iv). At the end of the second stage, the lyophilizer was brought to room temperature and the vacuum was broken with nitrogen. The chamber was purged with dry nitrogen for approximately 30 minutes before opening the door.

FIG. 2 is a SEM picture that shows a cross section of the foam with vertical channels. These channels run through the thickness of the foam.

Example 3

Architecturally Gradient Foam

This example describes the making of a foam that has a gradient in foam morphology as shown in FIG. 3 using a 10% solution of 35/65 ε-caprolactone-co-glycolide. The method used to make such a foam is similar to the description given in Example 2 with one difference. In step (ii) of the lyophilization process the time for which the solution is kept at the freezing step is 30 minutes.

FIG. 3 is a scanning electron micrograph of a cross section of this foam. Note the variation in the pore size and pore shape tbrough the thickness of the foam.

Example 4

Transcompositional Foam

This example describes-the making of a foam that has a compositional gradient and not necessarily a morphological gradient. Such a foam is made from polymer solutions that have been made from physical mixtures of two or more polymers. This example describes a transcompositional foam made from 35/65 PCL/PGA and 40/60 PCL/PLA.

Step A. Preparing a Solution Mixture of 35/65 PCL/PGA and 40/60 PCL/PLA in 1,4-Dioxane

In the preferred method the two separate solutions are first prepared (a) a 10% wt/wt polymer solution of 35/65 PCL/PGA and (b) a 10% wt/wt 40/60 PCL/PLA. Once tbese solutions are prepared as described in Example 1, equal parts of each solution was poured into one mixing flask. Tbe polymers used to make these solutions are described in Examples 8 and 9. A homogeneous solution of this physical mixture was obtained by gently heating to 60±5° C. and continuously stirring using a magnetic stir bar for approximately 2 hours.

Step B. Lyophilization Cycle

We used an FTS Dura Dry Freeze dryer with computer control and data monitoring system to make this foam. The first step in the preparation of such a foam was to generate a homogeneous solution as described in Step A. The solution was carefully filled into a dish just before the actual start of the cycle. The cylindrical glass dish weigbed 117 grams, was optical glass 2.5 mm thick and cylindrical with a 100 mm outer diameter and a 95 mm inner diameter. The lip height of the dish was 50 mm. Next the following steps were followed in sequence to make a 25 mm thick foam with the transcompositional gradient:

(i). The solution filled dish was placed on the freeze dryer shelf and the solution conditioned at 20° C. for 30 minutes. The cycle was started and the shelf temperature was set to −5° C. with a programmed cooling rate of 0.5° C./min.

(ii). The solution was held at the freezing condition (−5° C.) for 5 hours.

(iii). Vacuum was applied to initiate primary drying of the dioxane by sublimation and held at 100 milliTorr for 5 hours.

(iv). Next, secondary drying was done at 5° C. for 5 hours and at 20° C. for 10 hours. At each temperature the vacuum level was maintained at 20 mTorr.

(v). At the end of the second stage, the lyophilizer was brought to room temperature and the vacuum was broken with nitrogen. The chamber was purged with dry nitrogen for approximately 30 minutes before opening the door.

The foam has a gradient in chemical composition which is evident from a close scrutiny of the foam wall morphology as shown in FIGS. 4, 5 and 6. The gradient in the chemical composition was further supported by NMR data as detailed below:

Foam sample produced by the above method and which was approximately 25 mm thick was characterized for mole % composition. The foam sample is composed of a physical blend of PCL/PLA and PCL/PGA. Slices of the foam sample were prepared and analyzed to confirm that the material was a compositional gradient. The sample slices were identified as 1) foam IA (top slice), 2) foam IB (top middle slice), 3) foam IC (bottom middle slice), 4) foam ID (bottom slice). The NMR sample preparation consisted of dissolving a 5 mg of material into 300 μL hexafluoroacetone sesqua deutrium oxide (HFAD) and then diluting witb 300 μL of C_6D_6.

1H NMR Results: Mole % Composition

Sample ID	PLA	PGA	PCL
Foam IA	47.2	12.4	40.5
Foam IB	12.3	51.3	36.5
Foam IC	7.7	56.5	35.8
Foam ID	7.8	56.3	35.8

The NMR results indicate that the foam samples have a gradient with respect to composition. The top layer of the foam is high in PLA concentration (47 mole %), whereas the bottom layer of the foam is high in PGA concentration (56 mole %). These results suggest that the PCL/PGA copolymer and the PCL/PLA copolymer have differences in their phase separation behaviors during the freezing step and formed a unique compositionally gradient foam.

Example 5

Transstructural Foam

This example describes the making of a foam that has a compositional and structural gradient and not necessarily a morphological gradient. Such a foam is made from polymer solutions that have been made by physical mixtures of two or more polymers. This example describes a transcompositional foam made from 35/65 PCL/PLA (as described in Example 9) and 95/5 PLA/PCL (a random copolymer with an IV of 1.8 in HFIP measured as described herein). Note, 35/65 PCL/PLA is a soft elastomeric copolymer while 95/5 PLA/PCL is a relatively stiff copolymer. The combination of the two provides a compositional as well as structural gradient. This foam is made using the steps outlined in Example 4 starting from a homogeneous 50/50 physical mixture of a 10% wt./wt. solution of 35/65 PCL/PLA and 10% wt./wt. s Solution of 95/5 PLA/PCL in 1,4 dioxane. Such a transcompositional foam will provide a good template for tissue junctions such as bone-cartilage interfaces.

Example 6

Cell Culture and Differentiation Data

Films made from 95/5 PLA/PGA, 90/10 PGA/PLA, 95/5 PLA/PCL, 75/25 PGA/PCL and 40/60 PCL/PLA were tested. Tissue culture polystyrene (TCPS) was used as a positive control for all the assays. Before testing, polymer discs were positioned at the bottom of a 24-well ultralow cluster dish and were pre-wetted in growth media for 20 min.

The 95/5 PLA/PGA copolymer used in this example was a random copolymer with an IV of 1.76 as determined in HFIP at 25° C., which is currently used in Panacryl™ suture (Ethicon Inc., Somerville, N.J.). The 90/10 PGA/PLA copolymer was a random copolymer with an IV of 1.74 as determined in HFIP at 25° C., which is currently used in Vicyl™ suture (Ethicon Inc., Somerville, N.J.). The 95/5 PLA/PCL polymer was made as described in Example 10, with an IV of 2.1 as determined in HFIP at 25° C. The 75/25 PG/PCL copolymer is a segmented block copolymer with an IV of 1.85 and is described in U.S. Pat. No. 5,133,739 this copolymer is currently used in Monocry™ sutures (Ethicon Inc., Somerville, N.J.). The 40/60 PCL/PLA copolymer used in this Example was made as described in Example 9 and had an IV of 1.44.

Cell Attachment and Proliferation

Cells were seeded at 40,000/well in 24-well ultralow cluster dishes (Corning) containing the polymers. The ultralow cluster dishes are coatcd with a layer of hydrogel polymer, which retards protein and cell adhesion to the wells. Cell attachment to the biopolymers was determined following 24 hrs of incubation (N=3 for each polymer). The attached cells were released by trypsinization and the number of cells was determined using a heamacytometer. Cell proliferation was assessed by determining cell counts at days 3 and 6 following seeding.

Differentiation Assays

Alkaline Phosphatase Activity

Alkaline phosphatase activity was determined by a colorimetric assay using p-nitrophenol phosphate substrate (Sigma 104) and following manufacturers instruction. Briefly, cells were seeded on the films or meshes at a density of 40,000 cells/well and incubated for 1, 6, 14, and 21 d. Once cells reached confluence at day 6 they were fed with mineralization medium (growth medium supplemented with 10 mM β-glycerophosphate, 50 μg/ml ascorbic acid). Alkaline phosphatase activity was determined in cell homogenates (0.05% Triton X-100) at the above time points. The quantity of protein in cell extracts was determined by micro BCA reagent from Pierce. Primary rat osteoblasts cultured on films and meshes were also stained for membrane-bound alkaline phosphatase using a histochemical staining kit (Sigma). For all the films and meshes three samples per group were tested.

Osteocalin ELISA

Osteocalcin secreted into the medium by osteoblasts cultured on various films was quantified by ELISA (Osteocalcin ELISA kit, Biomedical Technologies Inc, Boston). Aliquots of media from the wells containing the polymer films were lyophilized prior to measurements of this protein by ELISA. Three samples for each polymer were tested and the ELISA was repeated twice.

Von Kossa Staining

Three samples for each polymer were stained for mineralized tissue using Von Kossa silver nitrate staining.

Expression of Alkaline Phosphatase and Osteocalcin mRNAs

The expression of alkaline phosphatase and osteocalcin mRNAs in cells was assessed by semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) using RNA extracted from cells cultured for 21 d on the films. Seven days after seeding, the culture media was replaced with mineralization media (3 mM β-glycerophosphate and 50 μg/ml of ascorbic acid were added). The cells were cultured for additional 2 weeks, for a total period of 3 weeks. Total RNA was extracted from four samples per group using a RNeasy mini kit provided by Qiagen. The quality and amount of total RNA was measured for each polymer group. Total RNA was reverse transcribed to obtain cDNA using a reverse transcriptase reaction (Superscript II, Gibco). The cDNAs for osteocalcin, alkaline phosphatase, and Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) were amplified using a PCR protocol described previously (GIBCO BRL manufacturers instruction). The primer sequences (Table I) for osteocalcin, alkaline phosphatase, and GAPDH were obtained using the FASTA program (Genetic Computer Group, Madison, Wis.). Preliminary studies were also conducted to optimize the number of PCR cycles for each primer (Table II), and to determine the range of RNA, which exhibits proportionality to cDNA. The PCR products were electrophoreses on 1% (wt) agarose gels containing ethidium bromide. The gels were photographed

under UV light and were evaluated by densitometry for the expression of osteocalcin and alkaline phosphatase mRNAs relative to GAPDH.

Statistical Anlysis

Analysis of variance (ANOVA) with Tukey post hoc comparisons was used to assess levels of significance for all the assays.

TABLE I

Primers used in RT-PCR

Gene	Species	Forward primer	Reverse primer	Size (bp)
Alkaline phosphatase	Rat	5' ATCGCCTATCAGCTAAT GCAC	5' GCAAGAAGAAGCCTTT GGG	379
Osteocalcin	Rat/Human	5'CAACCCCAATTGTGA CGAGC	5' TGGTGCGATCCATCAC AGAG	339
GAPDH	Mouse/Human/Rat	5'ACCACAGTCCATGCC ATCAC	5'TCCACCACCCTGTT GCTGTA	452

TABLE II

PCR optimization cycles

Gene	cDNA (μl)	Cycles
Alkaline phosphatase	1	25
Osteocalcin	1	35
GAPDH	1	23

Results

Cell attachment and proliferation on bioresorbable polymers: No observable difference in cell morphology was evident between the various polymer films and as compared to TCPS. Cell attachment to the various biopolymer films was equivalent to TCPS following 24 h of incubation. At day 3, cells proliferated well on all films with the exception of 40/60 PCL/PLA, where proliferation was 60% relative to TCPS. Furthermore, 95/5 PLA/PGA and 90/10 PGA/PLA films supported a significantly ($p<0.05$) higher degree of cell prolifcration compared to TCPS and 40/60 PCL/PLA (FIG. 7A).

Differentiation Assay

Alkaline Phosphatase Enzyme Activity

The profile for alkaline phosphatase activity expressed by osteoblasts cultured on 95/5 PLA/PGA, 90/10 PGA/PLA and 95/5 PLA/PCL films was similar to the profile observed on TCPS. Alkaline phosphatase specific activities were significantly ($p<0.05$) elevated for osteoblasts cultured on 40/60 PCL/PLA and 75/25 PGA/PCL films at days 14 and 21, respectively, compared to other films and TCPS (FIG. 7B).

Expression of Alkaline Phosphatase and Osteocalcin mRNA

The expression of mRNAs for alkaline phosphatase, osteocalcin, and GAPDH for osteoblasts cultured on the 95/5-PLA /PGA, 40/60 PLA/ PCL, 95/5 PLA/ PCL films, and TCPS were evaluated by densitometry. The results are depicted in FIG. 7C. It should be noted that the data in FIG. 7B is at best semi-quantitative. Nevertheless, the data suggests that 40/60 PCL/PLA film supported significantly ($p<0.05$) higher levels of osteocalcin expression compared to TCPS. The rest of the polymer surfaces were equivalent to TCPS for both osteocalcin and AP mRNAs expression.

Conclusions

No major differences were observed with respect to cell attachment and proliferation between the different bioresorbable films or meshes tested following 6 days of incubation. Furthermore, the results indicate that differences between these materials were more obvious with respect to their differentiation characteristics. Cells cultured on 40/60 PCL/PLA film showed enhanced alkaline phosphatase activity and osteocalcin mRNA expression compared to other films and TCPS following 14 and 21 days of incubation, respectively.

References that may be referred to for a more complete understanding of this techniques include, M. A. Aronow, L. C. Gerstenfeld, T. A. Owen, M. S. Tassinari, G. S. Stein and J. B. Lian: "Factors that promote progressive development of the osteoblast phenotype in cultured fetal rat calvaria cells: *Journal of Cellular Physiology*, 143: 213–221 (1990) and Stein, G. S., Lian, J. B., and Owen, t. A. "Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation" *FASEB*, 4, 3111–3123 (1990).

Example 7

In Vivo Study of Foam Blend in Swine Dermal Wound Healing Model

This example describes the results of implanting a 1 mm, 0.5 mm thickness foam tissue scaffolding in a swine full thickness excisional wound model. The foam tissue scaffold was made from a blend of 40/60 ε-caprolactone-co-lactide made as described in Example 8 and 35/65 ε-caprolactone-co-glycolide described in Example 9. These polymers were blended together and formed into 1 mm and 0.5 mm foams substantially as described in Example 3 (except that the cooling rate was 2.5° C. per minute and it was cooled only to −5° C.). Scanning electron micrographs of a 0.5 mm foam are presented in FIGS. 9A, 9B and 9C. The two thickness (0.5 mm and 1 mm) of foams were then tested in the wound excisional model with and without PDGF being provided. The resulting four different samples were then evaluated.

A blinded histologic evaluation was performed on 48 full thickness excisional wounds from four pigs (12 sites per animal) explanted at 8 days following wounding. The assessment was performed on H&E stained slides. During the histologic assessment, the following parameters were ranked/evaluated across the specimen set 1) cellular invasion of the matrix qualitative and quantitative assessments 2) infiltration of polymorphonuclear leukoctyes (PMNs) into the contact zone (ventral surface) of the matrix, 3) inflammation in the granulation tissue bed below (ventral to) the matrix, 4) reaction of the epidermis to the matrix, and 5) degree of fragmentation of the matrix.

Animal Husbandry

The pigs were housed individually in cages (with a minimum floor area of 10-sq. ft.) and given identification. All pigs were assigned an individual animal number. A tag was placed on each individual animal cage listing the animal number, species/strain, surgical date, surgical technique and duration of the experiment and date of euthanasia. Each animal was clearly marked with an animal number on the base of the neck using a permanent marker.

The animal rooms were maintained at the range of 40 to 70% R.H. and 15 to 24° C. (59.0 to 75.2° F). The animals were fed with a standard pig chow once per day, but were fasted overnight prior to any experimental procedure requiring anesthesia. Water was available ad libitum. A daily light:dark cycle of 12:12 hours was adopted.

Anesthesia

On the initial day of the study, days of evaluation and the day of necropsy, the animals were restrained and anesthetized with either an intramuscular injection of Tiletamine HCl plus Zolazepam HCl (Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa 4 mg/ml) and Xylazine (Rompun®, Bayer Corporation, Agriculture Division, Animal Health, Shawnee Mission, Kans., 4 mg/ml) or Isoflurane (AErrane Fort Dodge Animal Health, Fort Dodge, Iowa) inhalatory anesthesia (5% vol.) administered via a nose cone. When the animal was in the surgical suite, it was maintained on Isoflurane (AErrane®) inhalatory anesthesia (2% vol.) administered via a nose cone. Food was available after recovery from each procedure.

Preparation of the Surgical Site

One day prior to the surgical procedure, body weights were measured and the dorsal region of four pigs were clipped with an electric clipper equipped with a #40 surgical shaving blade. The shaved skin was then re-shaved closely with shaving cream and a razor and then rinsed. The shaved skin and entire animal (excluding the head) was then scrubbed with a surgical scrub brush-sponge with PCMX cleansing solution (Pharmaseal® Scrub Care® Baxter-Healthcare Corporation, Pharmaseal Division, Valencia, Calif.) and then with HIBICLENS® chlorhexidine gluconate (available from COE Laboratories, Incorporated, Chicago, Ill.). The animal was wiped dry with a sterile towel. Sterile NU-GAUZE* gauze (from Johnson & Johnson Medical Incorporated, Arlington, Tex.) was placed over the dorsal surface of each animal and secured with WATERPROOF* tape (available from Johnson & Johnson Medical Incorporated, Arlington, Tex.). The entire torso region of the animal was then wrapped with Spandage® elastic stretch bandage (available from Medi-Tech International Corporation, Brooklyn, N.Y.) to maintain a clean surface overnight.

On the day of surgery, immediately prior to delivering the animal to the surgical suite, the dorsal skin was again scrubbed using a surgical scrub brush-sponge with PCMX cleansing solution (Pharmaseal® Scrub Care®), rinsed and wiped dry using a sterile towel, as performed on the previous day. The animals were placed prone on the surgical table and wiped with 70% alcohol and dried with sterile gauze. Using a sterile surgical marker (availabe from Codman® a division of Johnson & Johnson Professional Incorporated, Raynham, Mass.) and an acetate template, marks were made on the dorsal skin according to the desired placement of each full-thickness wound.

Surgical Procedure

Following anesthesia, under sterile conditions, twelve (12) full-thickness excisions (1.5×1.5 cm) per animal were made in two rows parallel to the spinal column on the left and right dorsal regions using a scalpel blade. A pair of scissors and/or scalpel blade was used to aid in the removal of skin and subcutaneous tissue. Bleeding was controlled by use of a sponge tamponade. Sufficient space was left between wounds to avoid wound-to-wound interference. The excised tissue was measured for thickness using a digital caliper.

Application of the Treatment and Dressing

Each wound was submitted to a prepared, coded treatment regimen (study participants were blinded to all treatments). The primary dressing consisting of the sterile individual test article (1.5×1.5 cm soaked in sterile saline for 24 hours) was placed into the wound deficit in a predetermined scheme. The secondary dressing, a non-adherent, saline soaked, square of RELEASE* dressing (manufactured by Johnson & Johnson Medical Incorporated, Arlington, Tex.) was placed on top of the test article. A layer of BIOCLUSIVE* dressing (available from Johnson & Johnson Medical Incorporated, Arlington, Tex.) was sealed over the wounds to keep the wound moist and the dressing in place. Strips of Restonw (3M Medical-Surgical Division, St. Paul, Minn.) polyurethane self-adhering foam were placed between the wounds to avoid cross-contamination due to wound fluid leakage, and to protect the wounds from damage and the dressing from displacement. A layer of NU-GAUZE* gauze was placed on top of the BIOCLUSIVE* dressing and Reston™ foam, and was secured with WATERPROOF* tape to protect the dressings. The animals were then dressed with Spandage™ elastic net to help keep the dressings in place.

The secondary dressings were removed and replaced daily with a fresh piece of saline soaked RELEASE* secondary dressing. The primary dressings (test articles) were not disturbed unless the unit was displaced or pushed out of the wound deficit.

Post-Operative Care and Clinical Observations

After performing the procedures under anesthesia, the animals were returned to their cages and allowed to recover. The animals were given analgesics (buprenorphine hydrochloride [Buprenex Injectable, 0.01 mg/kg, im] sold by Reckitt & Colman Products, Hull, England) immediately post-surgery and the following day. After recovering from anesthesia, the pigs were observed for behavioral signs of discomfort or pain. No signs of pain were observed.

Each pig was observed twice daily after the day of surgery to determine its health status on the basis of general attitude and appearance, food consumption, fecal and urinary excretion and presence of abnormal discharges.

Euthanasia

At the end of the study (8 days post-wounding), each animal was euthanized under anesthesia, with an intravenous injection of (1 ml/10 pounds body weight) Socumb™ pentobarbital sodium and phenytoin sodium euthanasia solution (sold by The Butler Company, Columbus, Ohio) via the marginal ear vein. Following euthanasia, the animals were observed to ensure that respiratory function had ceased and there was no palpable cardiac function. A stethoscope facilitated the assessment for the lack of cardiac function.

Tissue Harvesting

Immediately following euthanasia, each wound, together with the underlying fat and a small portion of surrounding skin was excised. The tissue was placed in 10% neutral buffered formalin.

Evaluations

Visual Wound Assessment

General observations were recorded for days 1–3, including displacement, wound reaction and physical characteristics of the scaffold. Detailed clinical evaluations were performed on days 4–8 post-wounding. Assessments were

recorded as to the presence/absence (yes=1/no=0) and/or degree (given a score) of the following parameters:

Dressing Conditions: air exposed, displacement of test article, channeling, communication and moisture content of the RELEASE* secondary dressing(scored as: 4=moist, 3=moist/dry, 2=dry/moist, 1=dry).

Wound Bed Conditions: moisture content of test article (scored as: 4=moist, 3=moist/dry, 2=dry/moist, 1=dry), inflammation (scored as: 3=severe, 2=moderate, 1=slight, 0=none), reinjury (scored as: 3=severe, 2=moderate, 1=slight, 0=none), clots, folliculitis, infection, level of test article (scored as: 4=super raised, 3=raised, 2=even, 1=depressed), fibrin (scored as: 3=severe, 2=moderate, 1=slight, 0=none), and erythema. Color of the test article was also observed.

Tissue Processing

Excised tissue samples were taken at day eight. The entire wound was harvested and placed into 10% neutral buffered formalin. The tissue was prepared for frozen sections. The tissue was trimmed and mounted onto the object holder with Tissue-Tek® OCT Compound (sold by Sakura Finetechnical Company, Limited, Tokyo, Japan) and quickly frozen. The specimens were sectioned on the cryostat at 10 μm and stained with a frozen H&E stain.

Histological Assessments (Day 8 Post-Wounding)

Histological evaluations for granulation tissue (area and length) and epithelialization were assessed using H&E stained specimens using a magnification of 20–40×. Granulation tissue height was determined by dividing the area by the length.

Histopathological evaluation of the tissue samples was assessed using the H&E stained specimens, they were first assessed under 100× to 400×magnification.

Results

There was cellular invasion into the interstices of the matrix in the majority of all test sites. In the majority of sites this invasion was true tissue ingrowth comprised of varying populations of fibroblasts, macrophages, macrophage giant cells, and endothelial-like cells, there also appeared to be capillary formation. Significant formation of dense fibrous connective tissue layer dorsal to the matrices essentially embedding the matrices in the tissue, was seen at several sites for the 0.5 mm foams with and without PDGF. The 1 mm matrices were either at the surface of the tissue bed or sloughed. Macrophage giant cell formation seemed to be greater in the 0.5 mm versus the 1 mm foam scaffolds. In sites where the 1 mm foam was being sloughed or partially separated from the underlying granulation tissue there was death of the invading cells forming masses of pyknotic cell debris.

Complete incorporation of the matrix into the granulation tissue bed was seen at several sites for the 0.5 mm foam scaffoldings. FIGS. **10** and **11** illustrate the incorporation of these matrices into the granulation tissue bed. FIG. **10** is a dark filed 40× pictomicrograph of a trichrome stained tissue sample. FIG. **11** is a 100× composite photomicrograph of a trichrome stained sample illustrating the cellular invasion of a foam containing PDGF. Complete incorporation of the matrices into the granulation tissue bed is evident in both pictures. The dense fibrous tissue above the foam scaffolding is evident in both pictures. These results indicate the 0.5 mm foams will provide a suitable substrate for the growth of epidermal tissue.

Example 8

Synthesis of a Random Poly(ε-caprolactone-co-glycolide)

A random copolymer of ε-caprolactone-glycolide with a 35/65 molar composition was synthesized by ring opening polymerization reaction. The method of synthesis was essentially the method described in U.S. Pat. 5,468,253 in Example 6 (which is hereby incorporated herein by reference). The amount of dicthylene glycol initiator added was adjusted to 1.15 mmole/mole of monomer to obtain the following characteristics of the dried polymer: The inherent viscosity (I.V.) of the copolymer was 1.59 dL/g in hexafluoroisopropanol at 25° C. The molar ratio of PCL/PGA was found to be 35.5/64.5 by proton NMR with about 0.5% residual monomer. The glass transition (Tg) and the melting points (Tm) of the copolymer were found to be −1° C., 60° C. and 126° C. respectively, by DSC.

Example 9

Synthesis of 40:60 Poly(ε-caprolactone-co-L-lactide) by Sequential Addition

In the glove box, 100 μL (33 μmol) of a 0.33 M stannous octoate solution in toluene, 115 μL (1.2 mmol) of diethylene glycol, 24.6 grams (170 mmol) of L-lactide, and 45.7 grams (400 mmol) of ε-caprolactone were transferred into a silanized, flame dried, two neck, 250 mL round bottom flask equipped with a stainless steel mechanical stirrer and a nitrogen gas blanket. The reaction flask was placed in an oil bath already set at 190° C. and held there. Meanwhile, in the glove box, 62.0 grams (430 mmol) L-lactide were transferred into a flame dried, pressure equalizing addition funnel. The funnel was wrapped with heat tape and attached to the second neck of the reaction flask. After 6 hours at 190° C., the molten L-lactide was added to the reaction flask over 5 minutes. The reaction was continued overnight for a total reaction time of 24 hours at 190° C. The reaction was allowed to cool to room temperature overnight. The copolymer was isolated from the reaction flask by freezing in liquid nitrogen and breaking the glass. Any remaining glass fragments were removed from the copolymer using a bench grinder. The copolymer was again frozen with liquid nitrogen and broken off the mechanical stirring paddle. The copolymer was ground into a tared glass jar using a Wiley Mill and allowed to warm to room temperature in a vacuum oven overnight. 103.13 grams of 40:60 poly(ε-caprolactone-co-L-lactide) were added to a tared aluminum pan and then devolitilized under vacuum at 110° C. for 54 hours. 98.7 grams (95.7% by weight) of copolymer were recovered after devolitilization. The inherent viscosity was measured and found to be 1.1 dL/g in $CHCl_3$ at 25° C. (c=0.1 g/dL). FTIR (cast film from $CHCl_3$ onto KBr window, cm^{-1}): 2993, 2944, 2868, 1759, 1456, 1383, 1362, 1184, 1132, 1094, 870, and 756. ^1H NMR (400MHz, HFAD/Benzene, ppm): δ1.25, 2 broad lines (e); 1.35, 2 lines (f); 1.42, 3 lines; 1.55, 2 lines; 2.22, 3 lines ; 2.35, 4 broad lines; 4.01, 3 lines; 4.05, 3 lines; 4.2, quartet; 5.05, 3 broad lines; 5.15, 4 lines. Polymer composition by ^1H NMR: 41.8% PCL, 57.5% PLA, 0.8% L-lactide, <0.1% ε-caprolactone. DSC (20° C./min, first heat): T_m=154.8° C., ΔH_m=18.3 J/g. GPC (molecular weights determined in THF using poly(methyl methacrylate) standards, daltons): M_w=160,000, M_n=101,000, PDI=1.6.

Example 10

Synthesis of 95/5 PLA/PCL Copolymer

In the glove box, 170 μL (1.8 mmol) of diethylene glycol, 350 μL (115 μmol) of a 0.33 M stannous octoate solution in toluene, 17.1 grams (150 mmol) of ε-caprolactone, and 410.4 grams (2.85 mol) of L-lactide were placed into a silanized, flame dried, 1000 mL round bottom equipped with

a stainless steel mechanical stirrer and vacuum take off connector in order to maintain a dry nitrogen gas blanket. The reaction flask was placed in an oil bath already heated to 185° C. and then held there for 3 hours. The flask was removed from the oil bath and allowed to cool down to room temperature. The polymer was isolated by wrapping the flask with aluminum foil, freezing it in liquid nitrogen, and then grinding away any adhered glass to the polymer. The copolymer was then ground in a Wiley mill. The ground polymer was vacuum dried at 80° C. for 24 hours. 302 grams of copolymer were k collected. The inherent viscosity was 2.1 dL/g in chloroform [25° C., c=0.1 g/dL]. The copolymer composition was measured by proton NMR spectroscopy and found to be 97.2 mole percent PLA and 2.8 mole percent PCL. No residual monomer was detected.

We claim:

1. A method for the repair or regeneration of tissue comprising contacting cells with a biocompatible gradient foam having a biocompatible gradient foam having a first location and a second location wherein the biocompatible gradient foam has a substantially continuous transition in at least one characteristic selected from the group consisting of composition, stiffness, flexibility, bioabsorption rate and pore architecture from the first location to the second location of said biocompatible gradient foam.

2. The method of claim 1 wherein the biocompatible gradient foam is bioabsorbable.

3. The method of claim 1 wherein the biocompatible gradient foam is made from a bioabsorbable polymer selected from the group consisting of aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylenes oxalates, polyamides, poly(iminocarbonates), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups poly(anhydrides), polyphosphazenes, biopolymers and blends thereof.

4. The method foam of claim 3 wherein the bioabsorable polymer is an aliphatic polyester.

5. The method foam of claim 4 whercin the aliphatic polyester is selected from the group consisting of homopolymers and copolymers of lactide, lactic acid, glycolide, glycolic acid), ϵ-caprolactone, p-dioxanone (1,4-dioxan-2-one), trimethylene carbonate (1,3-dioxan-2-one), alkyl derivatives of trimethylene carbonate, δ-valerolactone, β-butyrolactone, γ-butyrolactone, ϵ-decalactone, hydroxybutyrate, hydroxyvalerate, 1,4-dioxepan-2-one, 1,5,8,12-tetraoxacyclotetradecane-7,14-dione), 1,5-dioxepan-2-one, 6,6-dimethyl-1,4-dioxan-2-one and polymer blends thereof.

6. The biocompatible gradient foam of claim 5 wherein the aliphatic polyester is an elastomer.

7. The method of claim 1 wherein cells are seeded onto the biocompatible gradient foam.

8. The method of claim 5 wherein cells are seeded onto the biocompatible gradient foam.

9. The method of claim 1 wherein the biocompatible gradient foam is implanted in an animal and contacted with cells.

10. The method of claim 5 wherein the biocompatible gradient foam is implanted in an animal and contacted with cells.

11. The method of claim 1 wherein the biocompatible gradient foam is seeded with cells and the biocompatible gradient foam and cells are placed in a cell culturing device and the cells are allowed to multiply on the biocompatible gradient foam.

12. The method of claim 5 whcrein the biocompatible gradient foam is seeded with cells and the biocompatible gradient foam and cells are placed in a cell culturing device and the cells are allowed to multiply on the biocompatible gradient foam.

13. The method of claim 5 wherein the cells are selected from the group consisting of pluripotent cells, stem cells, precursor cells and combinations thereof.

14. The method of claim 5 wherein the cells are selected from the group consisting of myocytes, adipocytes, fibromyoblasts, ectodermal cell, muscle cells, osteoblast, chondrocyte, endothelial cells, fibroblast, pancreatic cells, hepatocyte, bile duct cells, bone marrow cells, neural cells, genitourinary cells and combinations thereof.

15. The method of claim 7 wherein the cells are selected from the group consisting of pluripotent cells, stem cells, precursor cells and combinations thereof.

16. The method of claim 7 wherein the cells are selected from the group consisting of myocytes, adipocytes, fibromyoblasts, ectodermal cell, muscle cells, osteoblast, chondrocyte, endothelial cells, fibroblast, pancreatic cells, hepatocyte, bile duct cells, bone marrow cells, neural cells, genitourinary cells and combinations thereof.

17. The method of claim 1 wherein the biocompatible gradient foam contains an agent selected from the group consisting of antiinfectives, hormones, analgesics, anti-inflammatory agents, growth factors, chemotherapeutic agents, anti-rejection agents, prostaglandins, RDG peptides and combinations thereof.

18. A method for the repair or regeneration of tissue comprising contacting cells with a biocompatible foam having a first surface and a second surface with interconnecting pores and channels.

19. The method of claim 18 wherein the biocompatible foam is bioabsorbable.

20. The method of claim 18 wherein the biocompatible foam is made from a bioabsorbable polymer selected from the group consisting of aliphatic polyesters, poly(amino acids), copoly(ether-esters), polyalkylenes oxalates, polyamides, poly(iminocarbonatcs), polyorthoesters, polyoxaesters, polyamidoesters, polyoxaesters containing amine groups poly(anhydrides), polyphosphazenes, biopolymers and blends thereof.

21. The method of claim 20 wherein the bioabsorable polymer is an aliphatic polyester.

22. The method of claim 21 wherein the aliphatic polyester is selected from the group consisting of homopolymers and copolymers of lactide, lactic acid, glycolide, glycolic acid), ϵ-caprolactone, p-dioxanone (1,4-dioxan-2-one), trimethylene carbonate (1,3-dioxan-2-one), alkyl derivatives of trimethylene carbonate, δ-valerolactone, β-butyrolactone, γ-butyrolactone, ϵ-decalactone, hydroxybutyrate, hydroxyvalerate, 1,4-dioxepan-2-one, 1,5,8,12-tetraoxacyclotetradecane-7,14-dionc), 1,5-dioxepan-2-onc, 6,6-dimethyl-1,4-dioxan-2-one and polymer blends thereof.

23. The method of claim 22 wherein the aliphatic polyester is an elastomcr.

24. The method of claim 18 wherein cells are seeded onto the biocompatible foam.

25. The method of claim 22 wherein cells are seeded onto the biocompatible foam.

26. The method of claim 18 wherein the biocompatible foam is implanted in an animal and contacted with cells.

27. The method of claim 22 wherein the biocompatible foam is implanted in an animal and contacted with cells.

28. The method of claim 18 wherein the biocompatible foam is seeded with cells and the biocompatible foam and cells are placed in a cell culturing device and the cells are allowed to multiply on the biocompatible foam.

29. The method of claim 22 wherein the biocompatible foam is seeded with cells and the biocompatible foam and cells are placed in a cell culturing device and the cells are allowed to multiply on the biocompatible foam.

30. The method of claim 22 wherein the cells are selected from the group consisting of pluripotent cells, stem cells, precursor cells and combinations thereof.

31. The method of claim 22 wherein the cells are selected from the group consisting of myocytes, adipocytes, fibromyoblasts, ectodermal cell, muscle cells, osteoblast, chondrocyte, endothelial cells, fibroblast, pancreatic cells, hepatocyte, bile duct cells, bone marrow cells, neural cells, genitourinary cells and combinations thereof.

32. The method of claim 24 wherein the cells are selected from the group consisting of pluripotent cells, stem cells, precursor cells and combinations thereof.

33. The method of claim 24 wherein the cells are selected from the group consisting of myocytes, adipocytes, fibromyoblasts, ectodermal cell, muscle cells, osteoblast, chondrocyte, endothelial cells, fibroblast, pancreatic cells, hepatocyte, bile duct cells, bone marrow cells, neural cells, genitourinary cells and combinations thereof.

34. The method of claim 18 wherein the biocompatible foam contains an agent selected from the group consisting of antiinfectives, hormones, analgesics, anti-inflammatory agents, growth factors, chemotherapeutic agents, anti-rejection agents, prostaglandins, RDG peptides and combinations thereof.

35. The method of claim 11 wherein after the cells are allowed to multiply on the biocompatible foam, the biocompatible foam and the cells are implanted into an animal.

36. The method of claim 28 wherein after the cells are allowed to multiply on the biocompatible foam, the biocompatible foam and the cells are implanted into an animal.

* * * * *

US005863531A

United States Patent [19]

Naughton et al.

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[45] Date of Patent: *Jan. 26, 1999

[54] **IN VITRO PREPARATION OF TUBULAR TISSUE STRUCTURES BY STROMAL CELL CULTURE ON A THREE-DIMENSIONAL FRAMEWORK**

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[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. Nos. 4,963,489; 5,160,490; 5,032,508; 5,266,480 and 5,443,950.

[21] Appl. No.: **487,749**

[22] Filed: **Jun. 7, 1995**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 254,096, Jun. 6, 1994, abandoned, which is a continuation-in-part of Ser. No. 131,361, Oct. 4, 1993, Pat. No. 5,443,950, which is a division of Ser. No. 575,518, Aug. 30, 1990, Pat. No. 5,266,480, which is a division of Ser. No. 402,104, Sep. 1, 1989, Pat. No. 5,032,508, which is a continuation-in-part of Ser. No. 242,096, Sep. 8, 1988, Pat. No. 4,963,489, which is a continuation-in-part of Ser. No. 38,110, Apr. 14, 1987, abandoned, which is a continuation-in-part of Ser. No. 36,154, Apr. 3, 1987, Pat. No. 4,721,096, which is a continuation of Ser. No. 853,569, Apr. 18, 1986, abandoned.

[51] **Int. Cl.**[6] C12N 5/00; C12N 11/08; C12N 11/04; C12N 5/08

[52] **U.S. Cl.** 424/93.7; 424/423; 435/174; 435/180; 435/182; 435/395; 435/398

[58] **Field of Search** 435/174, 180, 435/18.2, 240.23, 395, 398; 424/93.7, 423

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[57] **ABSTRACT**

A stromal cell-based three-dimensional cell culture system is provided whicb can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. The stromal cells along with connective tissue proteins naturally secreted by the stromal cells attach to and substantially envelope a framework composed of a biocompatible non-living material formed into a three-dimensional structure baving interstitial spaces bridged by the stromal cells. Living stromal tissue so formed provides support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of cells in culture and/or cultures implanted in vivo. When grown in this three-dimensional system, the proliferating cells mature and segregate properly to form components of adult tissues analogous to counterparts in vivo, wbicb can be utilized in the body as a corrective tissue. The three-dimensional cultures can be used to form tubular tissue structures, like those of the gastrointestinal and genitourinary tracts, as well as blood vessels; tissues for hernia repair and/or tendons and ligaments.

27 Claims, 1 Drawing Sheet

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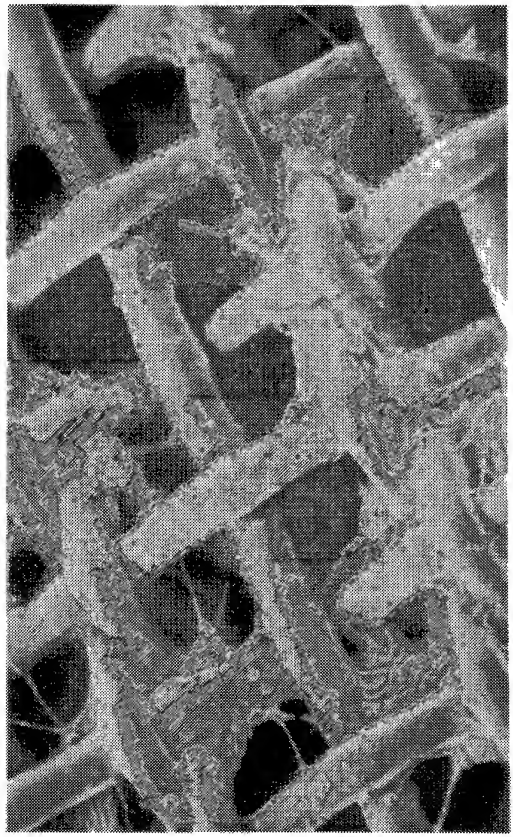

FIG. 1

IN VITRO PREPARATION OF TUBULAR TISSUE STRUCTURES BY STROMAL CELL CULTURE ON A THREE-DIMENSIONAL FRAMEWORK

The present application is a continuation-in-part of application Ser. No. 08/254,096 filed Jun. 6, 1994 (abandoned); which is a continuation-in-part of application Ser. No. 08/131,361 filed Oct.4, 1993 (U.S. Pat. No. 5,443,950); which is a divisional of application Ser. No. 07/575,518 filed Aug. 30, 1990 (U.S. Pat. No. 5,266,480); which is a divisional of application Ser. No. 07/402,104 filed Sep. 1, 1989 (U.S. Pat. No. 5,032,508); which is a continuation-in-part of application Ser. No. 242,096 filed Sep. 8, 1988 (U.S. Pat. No. 4,963,489); which is a continuation-in-part of application Ser. No. 038,110 filed Apr. 17, 1987 (abandoned); which is a continuation-in-part of application Ser. No. 036,154 filed Apr. 3, 1987 (U.S. Pat. No. 4,721,096); which is a continuation of application Ser. No. 853,569 filed Apr. 18, 1986 (abandoned), each of which is incorporated by reference herein in its entirety.

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1. INTRODUCTION

The present invention relates to a stromal cell-based three-dimensional cell and tissue culture system and its use

to form corrective structures that can be implanted and utilized in vivo. This culture system can be used for the long term proliferation of cells and tissues in vitro in an environment that more closely approximates that found in vivo. The culture system described herein provides for proliferation and appropriate cell maturation to form structures analogous to tissue counterparts in vivo. In particular, the invention relates to the use of the fibroblast-based three-dimensional cell culture system to construct complex structures such as, but not limited to, tubular sections of gastrointestinal and genitourinary tracts, blood vessels, tissues for hernia repair, tendons and ligaments. The three-dimensional cultures can be implanted in vivo to correct defects in the body.

2. BACKGROUND OF THE INVENTION

Cell culture systems have been used to study cells, expand cell populations for additional study, and in the production of recombinant gene products. However, cell culture systems have not been utilized for the repair of defects or abnormal tissues in the body.

2.1. Long Term Cell Culture

The majority of vertebrate cell cultures in vitro are grown as monolayers on an artificial substrate bathed in nutrient medium. The nature of the substrate on which the monolayers grow may be solid, such as plastic, or semisolid gels, such as collagen or agar. Disposable plastics have become the preferred substrate used in modern-day tissue or cell culture.

Some attempts have been made to use natural substrates related to basement membrane components. Basement membranes comprise a mixture of proteins, glycoproteins and proteoglycans that surround most cells in vivo. For example, Reid and Rojkund (1979, In, Methods in Enzymology, Vol. 57, Cell Culture, Jakoby & Pasten, eds., New York, Acad. Press, pp.263–278); Vlodavsky et al., (1980, Cell 19:607–617); Yang et al., (1979, Proc. Natl. Acad. Sci. USA 76:3401) have used collagen for culturing hepatocytes, epithelial cells and endothelial tissue. Growth of cells on floating collagen (Michalopoulos and Pitot, 1975, Fed. Proc. 34:826) and cellulose nitrate membranes (Savage and Bonney, 1978, Exp. Cell Res. 114:307–315) have been used in attempts to promote terminal differentiation. However, prolonged cellular regeneration and the culture of such tissues in such systems has not herctoforc been achieved.

Cultures of mouse embryo fibroblasts have been used to enhance growth of cells, particularly at low densities. This effect is thought to be due partly to supplementation of the medium but may also be due to conditioning of the substrate by cell products. In these systems, feeder layers of fibroblasts are grown as confluent monolayers which make the surface suitable for attachment of other cells. For example, the growth of glioma on confluent feeder layers of normal fetal intestine has been reported (Lindsay, 1979, Nature 228:80).

While the growth of cells in two dimensions is a convenient method for preparing, observing and studying cells in culture, allowing a high rate of cell proliferation, it lacks the cell-cell and cell-matrix interactions characteristic of whole tissue in vivo. In order to study such functional and morphological interactions, a few investigators have explored the use of three-dimensional substrates such as collagen gel (Douglas et al., 1980, In Vitro 16:306–312; Yang et al., 1979, Proc. Natl. Acad. Sci. 76:3401; Yang et al., 1980, Proc. Natl.

Acad. Sci. 77:2088–2092; Yang et al., 1981, Cancer Res. 41:1021–1027); cellulose sponge alone (Leighton et al., 1951, J. Natl. Cancer Inst. 12:545–561) or collagen coated (Leighton et al., 1968, Cancer Res. 28:286–296); a gelatin sponge, Gelfoam (Sorour et al., 1975, J. Neurosurg. 43:742–749).

In general, these three-dimensional substrates are inoculated with the cells to be cultured. Many of the cell types have been reported to penetrate the matrix and establish a "tissue-like" histology. For example, three-dimensional collagen gels have been utilized to culture breast epithelium (Yang et al., 1981, Cancer Res. 41:1021–1027) and sympathetic neurons (Ebendal, 1976, Exp. Cell Res. 98:159–169). Additionally, various attempts have been made to regenerate tissue-like architecture from dispersed monolayer cultures. Kruse and Miedema (1965, J. Cell Biol. 27:273) reported that perfused monolaycrs could grow to more than ten cells deep and organoid structures can develop in multilayered cultures if kept supplied with appropriate medium (see also Schneider et al., 1963, Exp. Cell Res. 30:449–459 and Bell et al., 1979, Proc. Natl. Acad. Sci. USA 76:1274–1279); Green (1978, Science 200:1385–1388) has reported that human epidermal keratinocytes may form dematoglyphs (friction ridges) if kept for several weeks without transfer; Folkman and Haudenschild (1980, Nature 288:551–556) reported the formation of capillary tubules in cultures of vascular endothelial cells cultured in the presence of endothelial growth factor and medium conditioned by tumor cells; and Sirica et al. (1979, Proc. Natl. Acad. Sci U.S.A. 76:283–287; 1980, Cancer Res. 40:3259–3267) maintained hepatocytes in primary culture for about 10–13 days on nylon meshes coated with a thin layer of collagen. However, the long term culture and proliferation of cells in such systems has not been achieved.

Indeed, the establishment of long term culture of tissues such as bone marrow has been attempted. Overall the results were disappointing, in that although a stromal cell layer containing different cell types is rapidly formed, significant hematopoiesis could not be maintained for any real time. (For review see Dexter et al., In Long Term Bone Marrow Culture, 1984, Alan R. Liss, Inc., pp. 57–96).

2.2. Correction of Defects in the Body

Surgical approaches to correcting defects in the body, in general, involve the implantation of structures made of biocompatible, inert materials, that attempt to replace or substitute for the defective function. Non-biodegradable materials will result in permanent structures that remain in the body as a foreign object. Implants that are made of resorbable materials are suggested for use as temporary replacements where the object is to allow the healing process to replace the resorbed material. However, these approaches have met with limited success for the long-term correction of structures in the body. For example, the use of a tubular mesh as a surgical corrective device is described in U.S. Pat. No. 4,347,847 of F. C. Usher issued Sep. 7, 1982. This mesh was used neither to generate a specific tissue culture, nor to reconstruct a tubular structure. Rather it was sutured in place in a flattened configuration to join connective tissues together. In U.S. Pat. No. 4,520,821, issued Jun. 4, 1985, Schmidt et al. disclose the use of a tubular mesh to correct defects in the tubular structures of the genitourinary tract.

The foreign meshes could not fully replace the damaged tissue, since smooth muscle does not grow at the treated site. Bell included a smooth muscle cell layer in his attempt at constructing blood vessels described in U.S. Pat. No. 4,546,500, issued Oct. 15, 1985. This construction, however, completely lacked elastin, a necessary component of blood vessels, and relied on a plastic mesh sleeve to provide the strength and elasticity required of blood vessels in vivo, with disappointing results. Thus, there has remained a need to construct tubular tissue structures (or constructs) such that they contain the cellular and extracellular components required to carry out the functions of their natural counterparts.

3. SUMMARY OF THE INVENTION

The present invention relates to a stromal cell-based three-dimensional cell culture system which can be used to culture a variety of different cells and tissues in vitro for prolonged periods of time. Growth of stromal cells on the three-dimensional framework results in the formation of a three-dimensional living stromal tissue which can be utilized in the body as a corrective structure. For example, and not by way of limitation, the three-dimensional cultures can be used to form tubular structures, like those of the gastrointestinal and genitourinary tracts, as well as blood vessels; tissues for hernia repair; tendons and ligaments; etc.

In accordance with the invention, stromal cells, such as fibroblasts, are inoculated and grown on a three-dimensional framework. The framework may be configured into the shape of the corrective structure desired. Stromal cells may also include other cells found in loose connective tissue such as smooth muscle cells, endothelial cells, macrophages/monocytes, adipocytes, pericytes, reticular cells found in bone marrow stroma, chondrocytes, etc. During growth in vitro the stromal cells deposit their extracellular matrix proteins onto the framework, thus forming a living stromal tissue; i.e., the stromal cells and connective tissue proteins naturally secreted by the stromal cells attach to and substantially envelope the framework composed of a biocompatible non-living material formed into a three-dimensional structure having interstitial spaces bridged by the stromal cells. The living stromal tissue so formed provides the support, growth factors, and regulatory factors necessary to sustain long-term active proliferation of cells in culture and deposition of appropriate matrix proteins. When grown in this three-dimensional system, the proliferating cells mature and segregate properly to form components of adult tissues analogous to counterparts found in vivo.

The invention is based, in part, on the discovery that growth of stromal cells in three dimensions will sustain active proliferation of cells in culture for longer periods of time than will monolayer systems. This may be due, in part, to the increased surface area of the three-dimensional framework which results in a prolonged period of active proliferation of stromal cells. These proliferating stromal cells elaborate proteins, growth factors and regulatory factors necessary to support the long term proliferation of both stromal and tissue-specific cells inoculated onto the stromal matrix. In addition, the three-dimensionality of the matrix allows for a spatial distribution which more closely approximates conditions in vivo, thus allowing for the formation of microenvironments conducive to cellular maturation and migration. The growth of cells in the presence of this support may be further enhanced by adding proteins, glycoproteins, glycosaminoglycans, a cellular matrix, and other materials to support itself or by coating the support with these materials. The three-dimensional framework can be shaped to assume the conformation of natural organs and their components.

In another embodiment of the invention, the stromal cells can be genetically engineered to express a gene product

beneficial for successful and/or improved transplantation. For example, in the case of vascular grafts, the stromal cells can be genetically engineered to express anticoagulation gene products to reduce the risk of thromboembolism, atherosclerosis, occlusion, or anti-inflammatory gene products to reduce risk of failure. For example, the stromal cells can be genetically engineered to express tissue plasminogen activator (TPA), streptokinase or urokinase to reduce the risk of clotting. Alternatively, the stromal cells can be engineered to express anti-inflammatory gene products, e.g., peptides or polypeptides corresponding to the idiotype of neutralizing antibodies for tumor necrosis factor (TNF), interleukin-2 (IL-2), or other inflammatory cytokines. Preferably, the cells are engineered to express such gene products transiently and/or under inducible control during the post-operative recovery period, or as a chimeric fusion protein anchored to the stromal cell, e.g., a chimeric molecule composed of an intracellular and/or transmembrane domain of a receptor or receptor-like molecule, fused to the gene product as the extracellular domain.

In another alternative, the stromal cells can be genetically engineered to "knock out" expression of factors or surface antigens that promote clotting or rejection. For example, expression of fibrinogen, von Willebrands factor or any cell surface molecule that binds to the platelet $\alpha 2B\beta$-3 receptor can be knocked out in the stromal cells to reduce the risk of clot formation. Likewise, the expression of MHC class II molecules can be knocked out in order to reduce the risk of rejection of the graft.

In yet another embodiment of the invention, the three-dimensional culture system of the invention may afford a vehicle for introducing genes and gene products in vivo to assist or improve the results of the transplantation and/or for use in gene therapies. For example, genes that prevent or ameliorate symptoms of vascular disease such as thrombus formation, atherosclerosis, inflammatory reactions, fibrosis and calcification, may be underexpressed or overexpressed in disease conditions. Thus, the level of gene activity in the patient may be increased or decreased, respectively, by gene replacement therapy by adjusting the level of the active gene product in genetically engineered stromal cells.

In another alternative, the stromal cells can be genetically engineered to block gene expression necessary for the transition of smooth muscle cells to proliferate, migrate and to lead to development of neointimal hyperplasia, e.g., by antisense oligodeoxynucleotide blockade of expression of cell division cycle 2 kinase and proliferating cell nuclear antigen. Mann, M. J., et al., 1995, Proc. Natl. Acad. Sci. USA 92:4502–4506.

The present invention relates to methods and biological tissue, tubular sections or constructs for the treatment, reconstruction and/or replacement of defects in the body, including, but not limited to, gastrointestinal and genitourinary tracts, blood vessels such as arteries and veins, tissues for hernia repair, tendons and ligaments.

3.1. Definition and Abbreviations

The following terms used herein shall have the meanings indicated:

Adherent Layer: cells attached directly to the three-dimensional support or connected indirectly by attachment to cells that are themselves attached directly to the support.

Stromal Cells: fibroblasts with or without other cells and/or elements found in loose connective tissue, including but not limited to, endothelial cells, pericytes, macrophages, monocytes, plasma cells, mast cells, adipocytes, etc.

Tissue-Specific or Parenchymal Cells: the cells which form the essential and distinctive tissue of an organ as distinguished from its supportive framework.

Three-Dimensional Framework: a three-dimensional scaffold composed of any material and/or shape that (a) allows cells to attach to it (or can be modified to allow cells to attach to it); and (b) allows cells to grow in more than one layer. This support is inoculated with stromal cells to form the living three-dimensional stromal tissue.

Three-Dimensional Stromal Tissue: a three-dimensional framework which has been inoculated with stromal cells that are grown on the support. The extracellular matrix proteins elaborated by the stromal cells are deposited onto the framework, thus forming a living stromal tissue. The living stromal tissue can support the growth of tissue-specific cells later inoculated to form the three-dimensional cell culture.

Three-Dimensional Cell Culture: a three-dimensional living stromal tissue which has been inoculated with tissue-specific cells and cultured. In general, the tissue specific cells used to inoculate the three-dimensional stromal matrix should include the "stem" cells (or "reserve" cells) for that tissue; i.e., those cells which generate new cells that will mature into the specialized cells that form the parenchyma of the tissue.

The following abbreviations shall have the meanings indicated:

BFU-E=burst-forming unit-erythroid
CFU-C=colony forming unit-culture
CFU-GEMM=colony forming unit-granuloid, erythroid, monocyte, megakaryocyte
EDTA=ethylene diamine tetraacetic acid
FBS=fetal bovine serum
HBSS=Hank's balanced salt solution
HS=horse serum
LTBMC=long term bone marrow culture
MEM=minimal essential medium
PBL=peripheral blood leukocytes
PBS=phosphate buffered saline
RPMI 1640=Roswell Park Memorial Institute medium number 1640 (GIBCO, Inc., Grand Island, N.Y.)
SEM=scanning electron microscopy

4. DESCRIPTION OF THE FIGURES

FIG. 1 is a scanning electron micrograph depicting attachment to the three-dimensional matrix and extension of cellular processes across the mesh opening. Fibroblasts are actively secreting matrix proteins and are at the appropriate stage of subconfluency which should be obtained prior to inoculant with tissue-specific cells.

5. DETAILED DESCRIPTION OF THE INVENTION: THE THREE-DIMENSIONAL CELL CULTURE SYSTEM

The present invention relates to three-dimensional living stromal tissues that can be used as corrective structures in the body, including, but not limited to, tubular structures that can be used to replace or repair blood vessels, gastrointestinal tract, or urinary tract; filamentous or tubular structures that can be used to replace or repair tendons and ligaments; and tubular or flat structures that can be used to repair defects such as hernias. The living stromal tissue of the invention comprises stromal cells grown on a three-dimensional framework, matrix or network. The three-dimensional framework can be formed into any desired shape; e.g., mesh type frameworks can be used to form tubular structures; rope-like structures can be woven or

tubes or filaments can be used as the framework for growing tendons and ligaments, etc.

In previously known tissue culture systems, the cells were grown in a monolayer. Cells grown on a three-dimensional stromal support, in accordance with the present invention, grow in multiple layers, forming a cellular matrix. This matrix system approaches physiologic conditions found in vivo to a greater degree than previously described monolayer tissue culture systems. The three-dimensional cell culture system is applicable to the proliferation of different types of cells and formation of a number of different tissues, including but not limited to bone marrow, skin, liver, pancreas, kidney, adrenal and neurologic tissue, as well as tissues of the gastrointestinal and genitourinary tracts, and the circulatory system, to name but a few. See U.S. Pat. Nos. 4,721,096; 4,963,489; 5,032,508; 5,266,480; and 5,160,490, each of which is incorporated by reference herein in its entirety.

The stromal cells used in the three-dimensional cultures comprise fibroblasts with or without additional cells and/or elements described more fully herein. The fibroblasts and other cells and/or elements that comprise the stroma may be fetal or adult in origin, and may be derived from convenient sources such as skin, liver, pancreas, arteries, veins, umbilical cord, and placental tissues, etc. Such tissues and/or organs can be obtained by appropriate biopsy or upon autopsy. In fact, cadaver organs may be used to provide a generous supply of stromal cells and elements.

Fetal fibroblasts will support the growth of many different cells and tissues in the three-dimensional culture system, and, therefore, can be inoculated onto the matrix to form a "generic" stromal support matrix for culturing any of a variety of cells and tissues. However, in certain instances, it may be preferable to use a "specific" rather than "generic" stromal support matrix, in which case stromal cells and elements can be obtained from a particular tissue, organ, or individual. For example, where the three-dimensional culture is to be used for purposes of transplantation or implantation in vivo, it may be preferable to obtain the stromal cells and elements from the individual who is to receive the transplant or implant. This approach might be especially advantageous where immunological rejection of the transplant and/or graft versus host disease is likely. Moreover, fibroblasts and other stromal cells and/or elements may be derived from the same type of tissue to be cultured in the three-dimensional system. This might be advantageous when culturing tissues in which specialized stromal cells may play particular structural/functional roles; e.g., smooth muscle cells of arteries, glial cells of neurological tissue, Kupffer cells of liver, etc.

Once inoculated onto the three-dimensional support, the stromal cells will proliferate on the framework and deposit the connective tissue proteins naturally secreted by the stromal cells. The stromal cells and their naturally secreted connective tissue proteins substantially envelop the framework thus forming the living stromal tissue which will support the growth of tissue-specific cells inoculated into the three-dimensional culture system of the invention. In fact, when inoculated with the tissue-specific cells, the three-dimensional stromal tissue will sustain active proliferation of the culture for long periods of time. Importantly, because openings in the mesh permit the exit of stromal cells in culture, confluent stromal cultures do not exhibit contact inhibition, and the stromal cells continue to grow, divide, and remain functionally active.

Growth and regulatory factors may be added to the culture, but are not necessary since they are elaborated by the stromal tissue. The use of growth factors (for example, but not limited to, αFGF, βFGF, insulin growth factor or TGF-betas), or natural or modified blood products or other bioactive biological molecules (for example, but not limited to, hyaluronic acid or hormones), even though not absolutely necessary in the present invention, may be used to further enhance the colonization of the three-dimensional framework or scaffolding.

Because, according to the invention, it is important to recreate, in culture, the cellular microenvironment found in vivo for a particular tissue, the extent to which the stromal cells are grown prior to use of the cultures in vivo may vary depending on the type of tissue to be grown in three-dimensional tissue culture. The living stromal tissues may be used as corrective structures by implanting them in vivo. Alternatively, the living stromal tissues may be inoculated with another cell type and implanted in vivo, with or without prior culturing in vitro. In addition, the stromal cells grown in the system may be genetically engineered to produce gene products beneficial to transplantation, e.g., anti-inflammatory factors, e.g., anti-GM-CSF, anti-TNF, anti-IL-1, anti-IL-2, etc. Alternatively, the stromal cells may be genetically engineered to "knock out" expression of native gene products that promote inflammation, e.g., GM-CSF, TNF, IL-1, IL-2, or "knock out" expression of MHC in order to lower the risk of rejection. In addition, the stromal cells may be genetically engineered for use in gene therapy to adjust the level of gene activity in a patient to assist or improve the results of the tubular tissue transplantation.

In another alternative, the stromal cells can be genetically engineered to block gene expression necessary for the transition of smooth muscle cells to proliferate, migrate and to lead to development of neointimal hyperplasia, e.g., by antisense oligodeoxynucleotide blockade of expression of cell division cycle 2 kinase and proliferating cell nuclear antigen.

The invention is based, in part, upon the discovery that growth of the stromal cells in three dimensions will sustain active proliferation of both the stromal and tissue-specific cells in culture for much longer time periods than will monolayer systems. Moreover, the three-dimensional system supports the maturation, differentiation, and segregation of cells in culture in vitro to form components of adult tissues analogous to counterparts found in vivo.

In yet another application, the three-dimensional tubular tissue or construct may be grown within a "bioreactor" to produce grafts populated with viable human cells. For example, but not limited to, a vascular graft, which may be assembled as a three-dimensional framework and housed in the treatment chamber of the bioreactor. Applying radial stress to the vascular graft located in the treatment chamber during seeding and culturing results in a vascular graft with cells and their fibers oriented so as to more likely tolerate the physiological conditions found in the human body. In this manner, the "bioreactor" creates a dynamic environment in which to seed and culture tissue-engineered vascular or other biological grafts or other implantable constructs.

Although the applicants are under no duty or obligation to explain the mechanism by which the invention works, a number of factors inherent in the three-dimensional culture system may contribute to its success:

(a) The three-dimensional framework provides a greater surface area for protein attachment, and consequently, for the adherence of stromal cells; and

(b) Because of the three-dimensionality of the framework, stromal cells continue to grow actively, in contrast to

cells in monolayer cultures, which grow to confluence, exhibit contact inhibition, and cease to grow and divide. The elaboration of growth and regulatory factors by replicating stromal cells may be partially responsible for stimulating proliferation and regulating differentiation of cells in culture;

(c) The three-dimensional framework allows for a spatial distribution of cellular elements which is more analogous to that found in the counterpart tissue in vivo;

(d) The increase in potential volume for cell growth in the three-dimensional system may allow the establishment of localized microenvironments conducive to cellular maturation;

(e) The three-dimensional framework maximizes cell-cell interactions by allowing greater potential for movement of migratory cells, such as macrophages, monocytes and possibly lymphocytes in the adherent layer;

(f) It has been recognized that maintenance of a differentiated cellular phenotype requires not only growth/differentiation factors but also the appropriate cellular interactions. The present invention effectively recreates the tissue microenvironment.

The three-dimensional stromal tissues, the culture system itself, and its maintenance, as well as various uses of the three-dimensional cultures are described in greater detail in the subsections below.

5.1. Establishment of the Three-Dimensional Stromal Tissue

The three-dimensional support or framework may be of any material and/or shape that: (a) allows cells to attach to it (or can be modified to allow cells to attach to it); and (b) allows cells to grow in more than one layer. A number of different materials may be used to form the framework, including but not limited to: non-biodegradable materials, e.g., nylon (polyamides), dacron (polyesters), polystyrene, polypropylene, polyacrylates, polyvinyl compounds (e.g., polyvinylchloride), polycarbonate (PVC), polytetrafluoroethylene (PTFE; teflon), thermanox (TPX), nitrocellulose, cotton; and biodegradable materials, e.g., polyglycolic acid (PGA), collagen, collagen sponges, cat gut sutures, cellulose, gelatin, dextran, polyalkanoates, etc. Any of these materials may be woven braided, knitted, etc., into a mesh, for example, to form the three-dimensional framework. The framework, in turn can be fashioned into any shape desired as the corrective structure, e.g., tubes, ropes, filaments, etc. Certain materials, such as nylon, polystyrene, etc., are poor substrates for cellular attachment. When these materials are used as the three-dimensional framework, it is advisable to pre-treat the framework prior to inoculation of stromal cells in order to enhance the attachment of stromal cells to the support. For example, prior to inoculation with stromal cells, nylon frameworks could be treated with 0.1M acetic acid, and incubated in polylysine, FBS, and/or collagen to coat the nylon. Polystyrene could be similarly treated using sulfuric acid.

For implantation of the three-dimensional culture in vivo, it may be preferable to use biodegradable matrices such as polyglycolic acid, collagen, collagen sponges, woven collagen, catgut suture material, gelatin, polylactic acid, or polyglycolic acid and copolymers thereof, for example. Where the cultures are to be maintained for long periods of time or cryopreserved, non-degradable materials such as nylon, dacron, polystyrene, polyacrylates, polyvinyls, teflons, cotton, etc., may be preferred. A convenient nylon mesh which could be used in accordance with the invention is Nitex, a nylon filtration mesh having an average pore size of 210 μm and an average nylon fiber diameter of 90 μm (#3-210/36, Tetko, Inc., N.Y.).

Stromal cells comprising fibroblasts, with or without other cells and elements described below, are inoculated onto the framework. These fibroblasts may be derived from organs, such as skin, liver, pancreas, etc., which can be obtained by biopsy (where appropriate) or upon autopsy. In fact fibroblasts can be obtained in quantity rather conveniently from any appropriate cadaver organ. As previously explained, fetal fibroblasts can be used to form a "generic" three-dimensional stromal matrix that will support the growth of a variety of different cells and/or tissues. However, a "specific" stromal tissue may be prepared by inoculating the three-dimensional framework with fibroblasts derived from the same type of tissue to be cultured and/or from a particular individual who is later to receive the cells and/or tissues grown in culture in accordance with the three-dimensional system of the invention.

Fibroblasts may be readily isolated by disaggregating an appropriate organ or tissue which is to serve as the source of the fibroblasts. This may be readily accomplished using techniques known to those skilled in the art. For example, the tissue or organ can be disaggregated mechanically and/or treated with digestive enzymes and/or chelating agents that weaken the connections between neighboring cells making it possible to disperse the tissue into a suspension of individual cells without appreciable cell breakage. Enzymatic dissociation can be accomplished by mincing the tissue and treating the minced tissue with any of a number of digestive enzymes either alone or in combination. These include but are not limited to trypsin, chymotrypsin, collagenase, elastase, and/or hyaluronidase, DNase, pronase, dispase etc. Mechanical disruption can also be accomplished by a number of methods including, but not limited to, the use of grinders, blenders, sieves, homogenizers, pressure cells, or insonators to name but a few. For a review of tissue disaggregation techniques, see Freshney, Culture of Animal Cells. A Manual of Basic Technique, 2d Ed., A. R. Liss,Inc., New York, 1987, Ch. 9, pp. 107–126.

Once the tissue has been reduced to a suspension of individual cells, the suspension can be fractionated into subpopulations from which the fibroblasts and/or other stromal cells and/or elements can be obtained. This also may be accomplished using standard techniques for cell separation including, but not limited to, cloning and selection of specific cell types, selective destruction of unwanted cells (negative selection), separation based upon differential cell agglutinability in the mixed population, freeze-thaw procedures, differential adherence properties of the cells in the mixed population, filtration, conventional and zonal centrifugation, centrifugal elutriation (counterstreaming centrifugation), unit gravity separation, countercurrent distribution, electrophoresis and fluorescence-activated cell sorting. For a review of clonal selection and cell separation techniques, see Freshney, Culture of Animal Cells. A Manual of Basic Techniques, 2d Ed., A. R. Liss, Inc., New York, 1987, Ch. 11 and 12, pp. 137–168.

The isolation of fibroblasts may, for example, be carried out as follows: fresh tissue samples are thoroughly washed and minced in Hanks balanced salt solution (HBSS) in order to remove serum. The minced tissue is incubated from 1–12 hours in a freshly prepared solution of a dissociating enzyme such as trypsin. After such incubation, the dissociated cells are suspended, pelleted by centrifugation and plated onto culture dishes. All fibroblasts will attach before other cells, therefore, appropriate stromal cells can be selectively iso-

lated and grown. The isolated fibroblasts can then be grown to confluency, lifted from the confluent culture and inoculated onto the three-dimensional matrix (see, Naughton et al., 1987, J. Med. 18 (3 and 4) 219–250). Inoculation of the three-dimensional framework with a high concentration of stromal cells, e.g., approximately 10 sup 6 to 5×10 sup 7 cells/ml, will result in the establishment of the three-dimensional stromal tissue in shorter periods of time.

In addition to fibroblasts, other cells may be added to form the three-dimensional stromal tissue. For example, other cells found in loose connective tissue may be inoculated onto the three-dimensional support along with fibroblasts. Such cells include but are not limited to smooth muscle cells, endothelial cells, pericytes, macrophages, monocytes, plasma cells, mast cells, adipocytes, etc. These stromal cells may readily be derived from appropriate organs such as arteries, skin, liver, etc., using methods known in the art such as those discussed above. In one embodiment of the invention, stromal cells which are specialized for the particular tissue to be cultured may be added to the fibroblast stroma. For example, stromal cells of hematopoietic tissue, including but not limited to fibroblasts, endothelial cells, macrophages/monocytes, adipocytes and reticular cells, could be used to form the three-dimensional subconfluent stroma for the long term culture of bone marrow in vitro. Hematopoietic stromal cells may be readily obtained from the "buffy coat" formed in bone marrow suspensions by centrifugation at low forces, e.g., 300×g. In the stromal layer that makes up the inner wall of arteries, a high proportion of undifferentiated smooth muscle cells can be added to provide the protein elastin. Stromal cells of liver may include fibroblasts, Kupffer cells, and vascular and bile duct endothelial cells. Similarly, glial cells could be used as the stroma to support the proliferation of neurological cells and tissues; glial cells for this purpose can be obtained by trypsinization or collagenase digestion of embryonic or adult brian (Ponten and Westermark, 1980, in Federof, S. Hertz, L., eds, "Advances in Cellular Neurobiology, "Vol. 1, New York, Academic Press, pp. 209–227). Again, where the cultured cells are to be used for transplantation or implantation in vivo it is preferable to obtain the stromal cells from the patient's own tissues. The growth of cells in the three-dimensional stromal cell culture may be further enhanced by adding to the framework, or coating the support with proteins (e.g., collagens, elastic fibers, reticular fibers) glycoproteins, glycosaminoglycans (e.g., heparan sulfate, chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate, keratin sulfate, etc.), a cellular matrix, and/or other materials.

The stromal cells may be inoculated onto the framework before or after forming the shape desired for implantation, e.g., tubes, ropes, filaments. After inoculation of the stromal cells, the three-dimensional framework should be incubated in an appropriate nutrient medium. Many commercially available media such as RPMI 1640, Fisher's, Iscove's, McCoy's, and the like may be suitable for use. It is important that the three-dimensional stromal cell cultures be suspended or floated in the medium during the incubation period in order to maximize proliferative activity. In addition, the culture should be "fed" periodically to remove the spent media, depopulate released cells, and add fresh media.

During the incubation period, the stromal cells will grow linearly along and envelop the three-dimensional framework before beginning to grow into the openings of the framework. It is important to grow the cells to an appropriate degree which reflects the amount of stromal cells present in the in vivo tissue prior to inoculation of the stromal matrix with the tissue-specific cells.

The openings of the framework should be of an appropriate size to allow the stromal cells to stretch across the openings. Maintaining actively growing stromal cells which stretch across the framework enhances the production of growth factors which are elaborated by the stromal cells, and hence will support long term cultures. For example, if the openings are too small, the stromal cells may rapidly achieve confluence but be unable to easily exit from the mesh; trapped cells may exhibit contact inhibition and cease production of the appropriate factors necessary to support proliferation and maintain long term cultures. If the openings are too large, the stromal cells may be unable to stretch across the opening; this will also decrease stromal cell production of the appropriate factors necessary to support proliferation and maintain long term cultures. When using a mesh type of support, as exemplified herein, we have found that openings ranging from about 150 μm to about 220 μm will work satisfactorily. However, depending upon the three-dimensional structure and intricacy of the framework, other sizes may work equally well. In fact, any shape or structure that allow the stromal cells to stretch and continue to replicate and grow for lengthy time periods will work in accordance with the invention.

Different proportions of the various types of collagen deposited on the support can also affect the growth of tissue-specific or other cells which may be later inoculated onto the stromal tissue or which may grow onto the structure in vivo. For example, for optimal growth of hematopoietic cells, the matrix should preferably contain collagen types III, IV and I in an approximate ratio of 6:3:1 in the initial matrix. For three-dimensional skin culture systems, collagen types I and III are preferably deposited in the initial matrix. The proportions of collagen types deposited can be manipulated or enhanced by selecting stromal cells which elaborate the appropriate collagen type and inoculating such stromal cells onto the framework. For example, fibroblasts can be selected using monoclonal antibodies of an appropriate isotype or subclass that is capable of activating complement, and which define particular collagen types. These antibodies and complement can be used to select negatively the fibroblasts which express the desired collagen type. Alternatively, the stroma used to inoculate the matrix can be a mixture of cells which synthesize the appropriate collagen types desired. The distribution and origins of the five types of collagen is shown in Table I.

TABLE I

DISTRIBUTIONS AND ORIGINS OF THE FIVE TYPES OF COLLAGEN

Collagen Type	Principal Tissue Distribution	Cells of Origin
I	Loose and dense ordinary connective tissue; collagen fibers	Fibroblasts and reticular cells; smooth muscle cells
	Fibrocartilage	
	Bone	Osteoblast
	Dentin	Odontoblasts
II	Hyaline and elastic cartilage	Chondrocytes
	Vitreous body of eye	Retinal cells

TABLE 1-continued

DISTRIBUTIONS AND ORIGINS OF THE FIVE TYPES OF COLLAGEN

Collagen Type	Principal Tissue Distribution	Cells of Origin
III	Loose connective tissue; reticular fibers	Fibroblasts and reticular cells
	Papillary layer of dermis	
	Blood vessels	Smooth muscle cells; endothelial cells
IV	Basement membranes	Epithelial and endothelial cells
	Lens capsule of eye	Lens fibers
V	Fetal membranes; placenta	Fibroblasts
	Basement membranes	
	Bone	
	Smooth muscle	Smooth muscle cells

Thus, depending upon the tissue to be cultured and the collagen types desired, the appropriate stromal cell(s) may be selected to inoculate the three-dimensional matrix.

Similarly, the relative amounts of collagenic and elastic fibers present in the stromal layer can be modulated by controlling the ratio of collagen producing cells to elastin producing cells in the initial inoculum. For example, since the inner walls of arteries are rich in elastin, an arterial stroma should contain a high concentration of the undifferentiated smooth muscle cells which elaborate elastin.

During incubation of the three-dimensional stromal cell cultures, proliferating cells may be released from the matrix. These released cells may stick to the walls of the culture vessel where they may continue to proliferate and form a confluent monolayer. This should be prevented or minimized, for example, by removal of the released cells during feeding, or by transferring the three-dimensional stromal culture to a new culture vessel. The presence of a confluent monolayer in the vessel will "shut down" the growth of cells in the three-dimensional matrix and/or culture. Removal of the confluent monolayer or transfer of the culture to fresh media in a new vessel will restore proliferative activity of the three-dimensional culture system. Such removal or transfers should be done in any culture vessel which has a stromal monolayer exceeding 25% confluency. Alternatively, the culture system could be agitated to prevent the released cells from sticking, or instead of periodically feeding the cultures, the culture system could be set up so that fresh media continuously flows through the system. The flow rate could be adjusted to both maximize proliferation within the three-dimensional culture, and to wash out and remove cells released from the culture, so that they will not stick to the walls of the vessel and grow to confluence. In any case, the released stromal cells can be collected and cryopreserved for future use.

The living stromal tissue so formed can be used as a corrective structure in vivo. Alternatively, other cells, such as parenchymal cells, may be inoculated and grown on the three-dimensional living stromal tissue prior to implantation in vivo.

5.2. Inoculation of Tissue-Specific Cells onto Three-Dimensional Stromal Matrix and Maintenance of Cultures

Once the three-dimensional stromal cell culture has reached the appropriate degree of growth, additional cells such as tissue-specific cells (parenchymal cells) or surface layer cells which are desired to be cultured may also be inoculated onto the living stromal tissue. Such cells inoculated onto the living stromal tissue can be incubated to allow the cells to adhere to the stromal tissue, and implanted in vivo where continued growth can occur. Alternatively, the cells can be grown on the living stromal tissue in vitro to form a cultured counterpart of the native tissue prior to implantation in vivo. A high concentration of cells in the inoculum will advantageously result in increased proliferation in culture much sooner than will low concentrations. The cells chosen for inoculation will depend upon the tissue to be cultured, which may include, but is not limited to, bone marrow, skin, liver, pancreas, kidney, neurological tissue, adrenal gland, mucosal epithelium, endothelium, and smooth muscle, to name but a few.

For example, and not by way of limitation, a variety of epithelial cells can be cultured on the three-dimensional living stromal tissue. Examples of such epithelial cells include, but are not limited to, oral mucosa and gastrointestinal (G.I.) tract cells. Such epithelial cells may be isolated by enzymatic treatment of the tissue according to methods known in the art, followed by expansion of these cells in culture and application of epithelial cells to the three-dimensional stromal support cell matrix (neo-submucosa). The presence of the submucosa provides growth factors and other proteins which promote normal division and differentiation of themoral mucosa cells and the cells of the G.I. tract lining. Using this methodology, other epithelial cells can be grown successfully, including nasal epithelium, respiratory tract epithelium, vaginal epithelium, and corneal epithelium.

In general, this inoculum should include of the "stem" cell (also called the "reserve" cell) for that tissue; i.e., those cells which generate new cells that will mature into the specialized cells that form the various components of the tissue.

The parenchymal or other surface layer cells used in the inoculum may be obtained from cell suspensions prepared by disaggregating the desired tissue using standard techniques described for obtaining stromal cells in Section 5.1 above. The entire cellular suspension itself could be used to inoculate the three-dimensional living stromal tissue. As a result, the regenerative cells contained within the homogenate will proliferate, mature, and differentiate properly on the matrix, whereas non-regenerative cells will not. Alternatively, particular cell types may be isolated from appropriate fractions of the cellular suspension using standard techniques described for fractionating stromal cells in Section 5.1 above. Where the "stem" cells or "reserve" cells can be readily isolated, these may be used to preferentially inoculate the three-dimensional stromal support. For example, when culturing bone marrow, the three-dimensional stroma may be inoculated with bone marrow cells, either fresh or derived from a cryopreserved sample. When culturing skin, the three-dimensional stroma may be inoculated with melanocytes and keratinocytes. When culturing liver, the three-dimensional stroma may be inoculated with hepatocytes. When culturing pancreas, the three-dimensional stroma may be inoculated with pancreatic endocrine cells. For a review of methods which may be utilized to obtain parenchymal cells from various tissues, see, Freshney, Culture of Animal Cells. A Manual of Basic Technique, 2d Ed., A. R. Liss, Inc., New York, 1987, Ch. 20, pp. 257–288.

During incubation, the three-dimensional cell culture system should be suspended or floated in the nutrient medium. Cultures should be fed with fresh media periodically. Again, care should be taken to prevent cells released from the

culture from sticking to the walls of the vessel where they could proliferate and form a confluent monolayer. The release of cells from the three-dimensional culture appears to occur more readily when culturing diffuse tissues as opposed to structured tissues. For example, the three-dimensional skin culture of the invention is histologically and morphologically normal; the distinct dermal and epidermal layers do not release cells into the surrounding media. By contrast, the three-dimensional bone marrow cultures of the invention release mature non-adherent cells into the medium much the way such cells are released in marrow in vivo. As previously explained, should the released cells stick to the culture vessel and form a confluent monolayer, the proliferation of the three-dimensional culture will be "shut down". This can be avoided by removal of released cells during feeding, transfer of the three-dimensional culture to a new vessel, by agitation of the culture to prevent sticking of released cells to the vessel wall, or by the continuous flow of fresh media at a rate sufficient to replenish nutrients in the culture and remove released cells. In any case, the mature released cells could be collected and cryopreserved for future use.

Growth factors and regulatory factors need not be added to the media since these types of factors are elaborated by the three-dimensional stromal cells. However, the addition of such factors, or the inoculation of other specialized cells may be used to enhance, alter or modulate proliferation and cell maturation in the cultures. The growth and activity of cells in culture can be affected by a variety of growth factors such as insulin, growth hormone, somatomedins, colony stimulating factors, erythropoietin, epidermal growth factor, hepatic erythropoietic factor (hepatopoietin), and liver-cell growth factor. Other factors which regulate proliferation and/or differentiation include prostaglandins, interleukins, and naturally-occurring chalones.

5.3. Uses of the Transplantable Tissue Grafts Grown in Three-Dimensional Culture System

The three-dimensional culture system of the invention can be used in a variety of applications. These include but are not limited to transplantation or implantation of either the cultured cells obtained from the matrix, or the cultured matrix itself in vivo. The three-dimensional tissue culture implants may, according to the invention, be used to replace or augment existing tissue, to introduce new or altered tissue, to modify artificial prostheses, or to join together biological tissues or structures. For example, and not by way of limitation, specific embodiments of the invention include but are not limited to: (i) dental prostheses joined to a three-dimensional culture of oral mucosa; (ii) tubular three-dimensional tissue implants (such as gastrointestinal tract, genitourinary tract and blood vessels; (iii) ligament or tendon implants; (iv) tissues for hernia repair; and (v) genetically altered cells grown in the three-dimensional culture which express a recombinant gene product.

5.3.1. Transplantation in Vivo

The three-dimensional cultures can be implanted in vivo to correct defects; replace surgically removed tissues; repair joints; implant shunts; repair hernias; etc. To this end, the living stromal tissue itself could be implanted in vivo. Depending upon the application, the implant may first be treated to kill the cells in the culture prior to implantation. For example, when treating conditions where growth factors may aggravate a pre-existing condition, e.g., in rheumatoid arthritis, it may be preferred to kill the cells which produce growth factors in the culture. This can be accomplished after the stromal tissue is formed in vitro but prior to implantation in vivo, by irradiation, or by freeze-thawing the cultures and washing away components of lysed cells.

Alternatively, where enhancement of wound healing is desired, the cultures can be implanted in a viable state so that growth factors are produced at the implant site. In yet another alternative, other cells, such as parenchymal cells, may be inoculated onto the living stromal tissue prior to implantation in vivo. These cultures may be further grown in vitro prior to implantation in vivo.

The basic manifestation of a hernia is a protrusion of the abdominal contents into a defect within the fascia. Surgical approaches toward hernia repair is focused on reducing the hernial contents into the peritoneal cavity and producing a firm closure of the fascial defect either by using prosthetic, allogeneic or autogenous materials. A number of techniques have been used to produce this closure including the movement of autologous tissues and the use of synthetic mesh products. Drawbacks to these current products and procedures include hernia recurrence, where the closure weakens again, allowing the abdominal contents back into the defect.

Insertion of the cultured invention in hernia repair would be likely via an open procedure despite trends toward minimally invasive surgeries as the conversion of herniorrhaphy from open to endoscopic procedures has proved slow.

In yet another example, ligaments and tendons are viscoelastic structures that increase in brittleness is with age, leading to ligamentous tears. These structures are complex, relatively static collagenous structures with functional links to the bone, muscle, menisci and other nearby tendons and ligaments. Surgical repair of these structures are conducted via either open procedures or arthroscopically-assisted procedures. Autografts are typically used from other sites in the knee. However, autografts can cause donor site morbidity. Other materials which are used in place of autografts, such as allografts, bovine tendons, polyesters and carbon fiber reinforced polymers, are subject to mechanical failure and can cause immunogenic complications.

5.3.2. Screening Effectiveness and Cytotoxicity of Compounds in Vitro

The three-dimensional cultures may be used in vitro to screen a wide variety of compounds, for effectiveness and cytotoxicity of pharmaceutical agents, growth/regulatory factors, natural and modified blood products, anticoagulants, clotting agents or anti-calcification agents, etc. To this end, the cultures are maintained in vitro and exposed to the compound to be tested. The activity of a cytotoxic compound can be measured by its ability to damage or kill cells in culture. This may readily be assessed by vital staining techniques. The effect of growth/regulatory factors may be assessed by analyzing the cellular content of the matrix, e.g., by total cell counts, and differential cell counts. This may be accomplished using standard cytological and/or histological techniques including the use of immunocytochemical techniques employing antibodies that define type-specific cellular antigens. The effect of various drugs on normal cells cultured in the three-dimensional system may be assessed.

5.3.3. Gene Therapy

The three-dimensional culture system of the invention may afford a vehicle for introducing genes and gene products in vivo to assist or improve the results of the transplantation and/or for use in gene therapies. For example, for

vascular grafts, the stromal cells can be genetically engineered to express anticoagulation gene products to reduce the risk of thromboembolism, or anti-inflammatory gene products to reduce the risk of failure due to inflammatory reactions. In this regard, the stromal cells can be genetically engincered to express TPA, streptokinase or urokinase to reduce the risk of clotting. Alternatively, for vascular or other types of tissuc grafts, thc stromal cells can be engineered to express anti-inflammatory gene products, for example, peptides or polypeptides corresponding to the idiotype of neutralizing antibodies for TNF, IL-2, or other inflammatory cytokines. Preferably, the cells are engineered to express such gene products transiently and/or under inducihle control during the post-operative recovery period, or as a chimeric fusion protein anchored to the stromal cells, for example, a chimeric molecule composed of an intracellular and/or transmembrane domain of a receptor or receptor-like molecule, fused to the gene product as the extracellular domain. In another embodiment, the stromal cells could be genetically engineered to express a gene for which a patient is deficient, or which would exert a therapeutic effect, e.g., HDL, apolipoprotein E, etc. The genes of interest engineered into the stromal cells need to be related to the disease being treated. For example, for vascular disease the stromal cells can be engineered to express gene products that are carried by the blood; e.g., cerebredase, adenosine deaminase, α-1-antitrypsin. In a particular embodiment, a genetically engineered vascular graft culture implanted to replace a section of a vein or artery can be used to deliver gene products such as α-1-antitrypsin to the lungs; in such an approach, constitutive expression of the gene product is preferred.

The stromal cells can be engineered using a recombinant DNA construct containing the gene used to transform or transfect a host cell which is cloned and then clonally expanded in the three-dimensional culture system. The three-dimensional culture which expresses the active gene product, could be implanted into an individual who is deficient for that product. For example, genes that prevent or ameliorate symptoms of various types of vascular, genitourinary tract, hernia or gastrointestinal diseases may be underexpressed or down regulated under disease conditions. Specifically, expression of genes involved in preventing the following pathological conditions may be down-regulated, for example: thrombus formation, inflammatory reactions, and fibrosis and calcification of the valves. Alternatively, the activity of gene products may be diminished, leading to the manifestations of some or all of the above pathological conditions and eventual development of symptoms of valvular disease. Thus, the level of gene activity may be increased by either increasing the level of gene product present or by increasing the level of the active gene product which is present in the three-dimensional culture system. The three-dimensional culture which expresses the active target gene product can then he implanted into the valvular disease patient who is deficient for that product. "Target gene," as used herein, refers to a is gene involved in diseases such as, but not limited to, vascular, genitourinary tract, hernia or gastrointestinal disease in a manner by which modulation of the level of target gene expression or of target gene product activity may act to ameliorate symptoms of valvular disease.

Further, patients may be treated hy gene replacement therapy during the post-recovery period after transplantation. Tissue constructs or sheets may he designed specifically to mcct the requirements of an individual patient, for example, the stromal cells may be genetically engineered to regulate one or more genes; or the regulation of gene expression may be transient or long-term; or the gene activity may be non-inducible or inducible. For example, one or more copies of a normal target gene, or a portion of the gene that directs the production of a normal target gene protein product with target gene function, may be inserted into human cells that populate the three-dimensional constructs using either non-inducible vectors including, but are not limited to, adenovirus, adeno-associated virus, and retrovirus vectors, or inducible promoters, including metallothionein, or heat shock protein, in addition to other particles that introduce DNA into cells, such as liposomes or direct DNA injection or in gold particles. For example, the gene encoding the human complement regulatory protein, which prevents rejection of the graft by the host, may be inserted into human fibroblasts. Nature 375:89 (May, 1995).

The three-dimensional cultures containing such genetically engineered stromal cells, e.g., either mixtures of stromal cells each expressing a different desired gene product, or a stromal cell engineered to express several specific genes are then implanted into the patient to allow for the amelioration of the symptoms of diseases such as, but not limited to, vascular, genitourinary tract, hernia or gastrointestinal disease. The gene expression may be under the control of a non-inducihle (i.e., constitutive) or inducible promoter. The level of gene expression and the type of gene regulated can be controlled depending upon the treatment modality being followed for an individual patient.

The use of the three-dimensional culture in gene therapy has a number of advantages. Firstly, since the culture comprises eukaryotic cells, the gene product will be properly expressed and processed in culture to form an active product. Secondly, gene therapy techniques are useful only if the numher of transfected cells can be substantially enhanced to be of clinical value, relevance, and utility; the three-dimensional cultures of the invention allow for expansion of the number of transfected cells and amplification (via cell division) of transfected cells.

A variety of methods may be used to obtain the constitutive or transient expression of gene products engineered into the stromal cells. For example, the transkaryotic implantation technique described by Seldon, R. F., et al., 1987, Science 236:714–718 can be uscd. "Transkaryotic", as used herein, suggests that the nuclei of the implanted cells have been altered by the addition of DNA sequences by stable or transient transfection. The cells can be engineered using any of the variety of vectors including, but not limited to, integrating viral vectors, e.g., retrovirus vector or adeno-associated viral vectors, or non-integrating replicating vectors, e.g., papilloma virus vectors, SV40 vectors, adenoviral vectors; or replication-defective viral vectors. Where transient expression is desired, non-integrating vectors and replication defective vectors may be preferred, since either inducible or constitutive promoters can be uscd in thcsc systems to control expression of the gene of interest. Alternatively, integrating vectors can be used to ohtain transient expression, provided the genc of intercst is controlled hy an inducihle promoter.

Preferahly, the expression control elements used should allow for the regulated expression of the gene so that the product is synthesized only when needed in vivo. The promoter chosen would depend, in part upon the type of tissue and cells cultured. Cells and tissues which are capable of secreting proteins (e.g., those characterized hy abundant rough endoplasmic reticulum, and golgi complex) are preferable. Hosts cells can hc transformcd with DNA controlled hy appropriate expression control elements (e.g., promoter,

enhancer, sequences, transcription terminators, polyadenylation sites, etc.) and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which, in turn, can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the gene protein product.

Any promoter may be used to drive the expression of the inserted gene. For example, viral promoters include but are not limited to the CMV promoter/enhancer, SV 40, papillomavirus, Epstein-Barr virus, elastin gene promoter and β-globin. If transient exprcssion is desired, such constitutive promoters are preferably used in a non-integrating and/or replication-defective vector. Alternatively, inducible promoters could be used to drive the expression of the inserted gene when necessary. For example, inducible promoters include, but are not limited to, metallothionein and heat shock protein.

Examples of transcriptional control regions that exhibit tissue specificity for connective tissues which have been described and could be used, include but are not limited to: elastin or elastase 1 gene control region which is active in pancreatic acinar cells (Swit et al., 1984, Cell 38:639–646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399–409; MacDonald, 1987, Hepatology 7:425–515). The deposition of elastin is correlated with specific physiological and developmental events in different tissues, including the vascular grafts. For example, in developing arteries, elastin deposition appears to be coordinated with changes in arterial pressure and mechanical activity. The transduction mechanisms that link mechanical activity to elastin expression involve cell-surface receptors. Once elastin-synthesizing cells are attached to elastin through cell-surface receptors, the synthesis of additional elastin and other matrix proteins may be influenced by exposure to stress or mechanical forces in the tissue (for example, the constant movement of the construct in the bioreactor) or other factors that influence cellular shape.

Once genetically engineered cells are implanted into an individual, the presence of TPA, streptokinase or urokinase activity can bring about amelioration of platelet aggregation, blood coagulation or thromboembolism. This activity is maintained for a limited time only, for example, to prevent potential complications that generally develop during the early phase after valve implantation, such as, platelet aggregation, blood clotting, coagulation or thromboembolism. Alternatively, once genetically engineered cells are implanted into an individual, the presence of the anti-inflammatory gene products, for example, peptides or polypcptidcs corresponding to the idiotypc of neutralizing antibodies for TNF, IL-2, or other inflammatory cytokines, can bring about amelioration of the inflammatory reactions associated with diseases such as vascular, gastrointestinal, hernia or genitourinary tract disease.

The stromal cells used in the three-dimensional culture system of the invention may be genetically engineered to "knock out" expression of factors or surface antigens that promote clotting or rejection at the implant site. Negative modulatory techniques for the reduction of target gene expression levels or target gene product activity levels are discussed below. "Negative modulation", as used herein, refers to a reduction in the level and/or activity of target gene product relative to the level and/or activity of the target gene product in the absence of the modulatory treatment. The expression of a gene native to stromal cell can be reduced or knocked out using a number of techniques, for example, expression may be inhibited by inactivating the gene completely (commonly termed "knockout") by using the homologous recombination technique. Usually, an exon encoding an important region of the protein (or an exon 5' to that region) is interrupted by a positive selectable marker (for example neo), preventing the production of normal mRNA from the target gene and resulting in inactivation of the gene. A gene may also be inactivated by creating a deletion in part of a gene, or by deleting the entire gene. By using a construct with two regions of homology to the target gene that are far apart in the genome, the sequences intervening the two regions can be deleted. Mombaerts, P., et al., 1991, Proc. Nat. Acad. Sci. U.S.A. 88:3084–3087.

Antisense and ribozyme molecules which inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene activity. For example, antisense RNA molecules which inhibit the expression of major histocompatibility gene complexes (HLA) shown to be most versatile with respect to immune responses. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. These techniques are described in detail by L. G. Davis, et al., eds, *Basic Methods in Molecular Biology,* 2nd ed., Appleton & Lange, Norwalk, Conn. 1994.

In another alternative, the stromal cells can be genetically engineered to block gene expression necessary for the transition of smooth muscle cells to proliferate, migrate and to lead to development of neointimal hyperplasia, e.g., by antisense oligodeoxynucleotide blockade of expression of cell division cycle 2 kinase and proliferating cell nuclear antigen. Mann, M. J., et al., 1995, Proc. Natl. Acad. Sci. USA 92:4502–4506.

Using any of the foregoing techniques, the expression of fibrinogen, von Willebrands factor, factor V or any cell surface molecule that binds to the platelet α2Bβ-3 receptor can be knocked out in the stromal cells to reduce the risk of clot formation in the vascular or other types of biological tissue grafts. Likewise, the expression of MHC class II molecules can be knocked out in order to reduce the risk of rejection of the graft.

In yet another embodiment of the invention, the three-dimensional culture system could be used in vitro to produce biological products in high yield. For example, a cell which naturally produces large quantities of a particular biological product (e.g., a growth factor, regulatory factor, peptide hormone, antibody, etc.), or a host cell genetically engineered to produce a foreign gene product, could be clonally expanded using the three-dimensional culture system in vitro. If the transformed cell excretes the gene product into the nutrient medium, the product may be readily isolated from the spent or conditioned medium using standard separation techniques (e.g., HPLC, column chromatography, electrophoretic techniques, to name but a few). A "bioreactor" has been devised which takes advantage of the flow method for feeding the three-dimensional cultures in vitro. Essentially, as fresh media is passed through the three-dimensional culture, the gene product is washed out of the culture along with the cells released from the culture. The gene product is isolated (e.g., by HPLC column chromatography, electrophoresis, etc.) from the outflow of spent or conditioned media.

The three-dimensional culture system of the invention may also afford a vehicle for introducing genes and gene

products in vivo for use in gene therapies or to augment healing at the site of implantation. For example, using recombinant DNA techniques, a gene for which a patient is deficient could he placed under the control of a viral or tissue-specific promoter. Alternatively, DNA encoding a gene product that enhances wound healing may be engineered into the cells grown in the three-dimensional system. The recombinant DNA construct containing the gene could he used to transform or transfect a host cell which is cloned and then clonally expanded in the three-dimensional culture system. The three-dimensional culture which expresses the active gene product, could be implanted into an individual who is deficient for that product.

The usc of the three-dimensional culture in gene therapy has a number of advantages. Firstly, since the culture comprises eukaryotic cells, the gene product will be properly expressed and processed in culture to form an active product. Secondly, gene therapy techniques are useful only if the number of transfected cells can be substantially enhanced to be of clinical value, relevance, and utility; the three-dimensional cultures of the invention allow for expansion of the number of transfected cells and amplification (via cell division) of transfected cells. For example, genetically engineered cells that express the gene product could be incorporated into living stromal tissue tubes that can be used as hlood vessels; in this case the gene product may be delivered to the bloodstream where it will circulate. Alternatively, genetically engineered cells that express wound healing factors may be incorporated into the living stromal cultures used to make tendons and ligaments to enhance wound healing at the site of implantation.

Preferahly, the expression control elements used should allow for the regulated expression of the gene so that the product is synthesized only when needed in vivo. The promoter chosen would depend, in part upon the type of tissue and cells cultured. Cells and tissues which are capable of secreting proteins (e.g., those characterized by abundant rough endoplasmic reticulum and golgi complex) are preferahle. To this end, liver and other glandular tissues could be selected. When using liver cells, liver specific viral promoters, such as hepatitis B virus elements, could be used to introduce foreign genes into liver cells and regulate the expression of such genes. These cells could then be cultured in the three-dimensional system of the invention. Alternatively, a liver-specific promoter such as the albumin promoter could be used.

Examples of transcriptional control regions that exhibit tissue specificity which have heen described and could be used, include but are not limited to: elastase 1 gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639–646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399–409; MacDonald, 1987, Hepatology 7:42–51S); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115–122); immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647–658; Adams et al., 1985, Nature 318:533–538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436–1444); albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268–276); alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639–1648; Hammer et al., 1987, Science 235:53–58); alpha-1-antitrypsin gene control region which is active in liver (Kelsey et al., 1987, Genes and Devel. 1:161–171); heta-globin gene control region which is active in mycloid cells (Magram et al., 1985, Nature 315:338–340; Kollias et al., 1986, Cell 46:89–94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703–712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Naturc 314:283–286); and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372–1378).

In a further embodiment of the invention, three-dimensional cultures maybe used to facilitate gene transduction. For example, and not by way of limitation, three-dimensional cultures of fibroblast stroma comprising a recombinant virus expression vector may be used to transfer the recombinant virus into cells brought into contact with the stromal matrix, thereby simulating viral transmission in vivo. The three-dimensional culture system is a more efficient way of accomplishing gene transduction than are current techniques for DNA transfection.

In an alternate embodiment of the invention, the three-dimensional cultures may be used as model systems for the study of physiologic or pathologic conditions and the effect of drugs and treatments. For example, in a specific embodiment of the invention, a three-dimensional culture system may be used as a model for the blood-brain barrier; such a model system can be used to study the penetration of substances through barriers such as the blood-brain barrier, the glomerular apparatus, and mucosa of nasopharyngeal passage lining.

For purposes of description only, and not by way of limitation, sample embodiments of the invention are described below. For purposes of description only, and not by way of limitation, the formation of the three-dimensional cultures into tubes is described hased upon the type of tissue and cells used in various systems. These descriptions specifically include but are not limited to tuhular sections of gastrointestinal tract, genitourinary tract as well as hlood vessels. It is expressly understood that the three-dimensional culture system can be used with other types of cells to form other types of tubular tissues, all of which tissues are encompassed by the invention.

6. TUBULAR BIOLOGICAL TISSUES

The three-dimensional culture system can he used to construct single and multi-layer tubular tissues in vitro. In accordance with the invention, these tubular structures can simulate tubular tissues and organs in the body, including, but not limited to, blood vessels, gastrointestinal tract and genitourinary tract.

The different biological structures described below have several features in common. They are all tubular structures primarily composed of layers of stromal tissue with an interior lining of epithelium (gastrointestinal and genitourinary) or endothelium (blood vessels). Their connective tissues also contain layers of smooth muscle with varying degrees of elastic fibers, both of which are especially prominent in arterial blood vessels. By including and sustaining these components in three-dimensional cultures according to the present invention, the tissues they compose can attain the special structural and functional properties they require for proper physiological functioning in vivo. They can then serve as replacements for damaged or diseased tubular tissues in a living body.

6.1. Single Mesh Layer Tubes

The following subsections describe the use of a mesh framework to support the growth of the living stromal tissue used to prepare tubes that can be implanted into the body.

6.1.1. Flat Mesh Starting Material

A mesh can be cut into a rectangular strip of which the width is approximately equal to the inner circumference of the tubular organ into which it will ultimately be inserted. The cells can be inoculated onto this mesh and incubated by floating or suspending in liquid media. At the appropriate stage of confluence, the mesh can be rolled up into a tube by joining the long edges together. The seam can be closed by suturing the two edges together using fibers of a suitable material of an appropriate diameter.

6.1.2. Tubular Mesh Starting Material

According to the invention, a mesh can be woven as a tube, inoculated with stromal cells and suspended in media in an incubation chamber. In order to prevent cells from occluding the lumen, one of the open ends of the tubular mesh can be affixed to a nozzle. Liquid media can be forced through this nozzle from a source chamber connected to the incubation chamber to create a current through the interior of the tubular mesh. The other open end can be affixed to an outflow aperture which leads into a collection chamber, from which the media can be recirculated through the source chamber. The tube can be detached from the nozzle and outflow aperture when incubation is complete. This method is described by Ballermann, B. J., et al., Int. Application No. WO 94/25584 and in a pending application entitled, "APPARATUS AND METHOD FOR STERILIZING, SEEDING, CULTURING, STORING, SHIPPING AND TESTING TISSUE, SYNTHETIC, OR NATIVE VASCULAR GRAFTS," filed Apr. 27, 1995 by Peterson, A., et al. (Advanced Tissue Sciences, Inc.), Ser. No. 08/430,768, both of which are incorporated herein by reference in its entirety.

6.2. Multiple Mesh Layers Tubes

In general, two three-dimensional cultures can be combined into a tube in accordance with the invention using any of the following methods.

6.2.1. Multiple Flat Meshes

One flat rectangular culture can be laid atop another and sutured together. This two-layer sheet can then be rolled up, as described above for a single culture in Section 6.1.1, by joining together the long edges, and securing with sutures.

6.2.2. Flat Meshes Wrapped Around Tubular Meshes

One tubular mesh that is to serve as the inner layer can be inoculated and incubated. A second mesh can be grown as a flat, rectangular strip with width slightly larger than the outer circumference of the tubular mesh. After appropriate growth is attained, the rectangular mesh can be wrapped around the outside of the tubular mesh. Closing the seam of the outer strip and securing it to the inner tube can be accomplished in a single suturing step.

6.2.3. Multiple Tubular Meshes

Two tubular meshes of slightly differing diameters can be grown separately. The culture with the smaller diameter can be inserted inside the larger one, and secured with sutures. This method would not be practical for very narrow tubes.

For each of these methods, more layers can be added by reapplying the method to the double layered tube. The meshes can be combined at any stage of growth of the culture they contain, and incubation of the combined meshes can be continued when desirable.

According to the present invention, any suitable method can be employed to shape the three-dimensional culture to take on the conformation of the natural organ or tissue to be simulated.

The descriptions which follow are provided to demonstrate how to construct model tubular tissues and organs in vitro. In each case, one or more stromal layers can be established as described in section 5.1. Particular attention is paid to generating the specialized properties of specific natural connective tissues by including and maintaining the materials inherent to those natural tissues in the three-dimensional cultures. One or more surface layers of generally more homogenous cellular composition (such as endothelium, epithelium, or smooth muscle) can then be cultured onto the stromal layer as described in Section 5.2. Using the methods outlined above in this section, these three-dimensional cultures can be shaped to assume a tubular conformation which simulates the shape of a natural tubular organ or tissue. Variations of this basic approach can be used to better simulate the natural organs and tissues to be corrected.

These tubular constructions simulate biological structures in vivo and may be readily implanted to replace damaged or diseased tissues. However, the invention encompasses the three-dimensional cultures described herein in any possible form and does not require that these cultures be formed into tubes. Flat three-dimensional cultures can be implanted, for example, directly into the body to replace any part or all of the circumference of a tubular structure, depending on the extent of replacement required.

7. BLOOD VESSELS

The replication of blood vessel elements in vitro is described below in particular for arteries and veins.

7.1. Arteries

Arteries are tubes lined with a thin layer of endothelial cells and generally composed of three layers of connective tissue: the intima (which is not present in many muscular arteries, particularly smaller ones), media, and adventitia, in order from inside to outside.

The main cellular component of the inner two layers is an undifferentiated smooth muscle cell, which produces the extracellular protein elastin. The internal elastic lamina, which lies just interior to the media, is a homogenous layer of elastin. The abundance of elastin in their walls gives arteries the ability to stretch with every contraction of the heart. The intima and media also contain some fibroblasts, monocytes, and macrophages, as well as some collagen.

The adventitia is composed of more ordinary connective tissue with both elastic and collagenic fibers. Collagen in this layer is important in preventing over-stretching.

While all the layers of the arterial wall are connective tissue, there is a compositional and functional difference between the adventitia and the inner two coats, the intima and the media. Consequently, it may be advantageous in accordance with the invention to grow these different layers in separate meshes. Whether the intima and media are grown in separate meshes, or combined in one, depends on how distinct these layers are in the particular artery into which the three-dimensional culture is to be implanted.

For example, according to the invention fibroblasts can be isolated from the adventitia of a patient's artery and used to inoculate a three-dimensional matrix, as described in Section 6.1, and grown to subconfluence. Cells can be isolated

from tissue rich in elastin-producing undifferentiated smooth muscle cells, also containing some fibroblasts, from the intima and media of the same artery. These cells can be used to inoculate a separate mesh and grown to subconfluence. Once the elastin-producing cells have proliferated to the appropriate extent, these meshes can be combined using one of the methods detailed in Section 6.2. In this manner, the smooth muscle cells can proliferate and produce elastin in a three-dimensional environment that simulates that of natural arterial walls.

Endothelial cells can be isolated from the same patient. When the two cultures reach the appropriate degree of confluence, the endothelial cells can be seeded on top of the upper, elastin-rich layer and incubated until they form a confluent layer.

If a fully functional replacement with all the various layers of tissue is not required, a simple homogenous three-dimensional elastin-rich stromal culture can be used. Alternatively, the stromal culture could be lined with endothelium. More layers of this homogenous stromal matrix can be combined to provide the appropriate thickness for such a prosthesis.

7.2. Veins

The layers of the connective tissue comprising the walls of veins are less well delineated than those of arteries, and contain much more collagen and less elastin. Consequently, a single three-dimensional culture can be grown, for example, from a single inoculum of cells. These cells consisting mostly of fibroblasts with some smooth muscle cells, can be isolated from the walls of a vein of the patient. When the appropriate degree of confluence is reached, endothelial cells, isolated from the same patient, for example, can be seeded on top of the stromal layer and grown to confluence.

8. GASTROINTESTINAL TRACT

Another embodiment of the invention provides for the replication of gastrointestinal tract elements in vitro in a system comparable to physiological conditions. The gastrointestinal tract comprises several different organs, but all have the same general histological scheme.

1. Mucous Membrane: The mucous membrane is the most interior layer of the gastrointestinal tract, and is composed of three sub-layers. The absorptive surfaces particularly are highly folded to increase the surface area. The lumen is lined with a thin layer of epithelium, which is surrounded by the lamina propria, a connective tissue which contains fibroblasts, some smooth muscle, capillaries, as well as collagenic, reticular, and some elastic fibers. Lymphocytes are also found here to protect against invasion, especially at absorptive surfaces where the epithelium is thin. The third sub-layer, the muscularis mucosa, consists of two thin layers of smooth muscle with varying amounts of elastic fibers. The smooth muscle fibers of the inner layer are arranged circularly, and the outer layer is arranged longitudinally.

2. Submucosa: This layer consists of loose connective tissue including elastic fibers as well as larger blood vessels and nerve fibers.

3. Muscularis Externa: This layer consists of two thick layers of smooth muscle which provide the motion which advances material along through the gastrointestinal tract. The muscle fibers of the inner layer are arranged circularly, while in the outer layer they are longitudinal. An exception is the upper third of the esophagus, which contains striated muscle allowing for the voluntary contractions associated with swallowing.

4. Serosa (or Adventitia): This outermost layer consists of loose connective tissue, covered by squamous mesothelium where the tract is suspended freely.

These four layers can be constructed in vitro in accordance with the invention by making different three-dimensional tubular tissue cultures. For example, in order to construct the mucous membrane, a mesh composed of bioabsorbable material can be inoculated with fibroblasts, smooth muscle cells, and other cells isolated from the lamina propria of the patient who is to receive the implant, from a section of tract in or around the site that is to be replaced.

If the site of transplantation is an absorptive surface, the mesh can be contoured on the surface which is to face the lumen.

Simultaneously, a second mesh whose inner circumference is slightly larger than the outer circumference of the first mesh can be inoculated with the fibroblasts and other cells of the patient's submucosal layer. Similarly, a third mesh can be inoculated with cells from the serosa. These meshes can be configured and incubated as outlined in section 7.1.

When each stromal layer has grown to the appropriate extent, the respective surface layers can be cultured. For example, epithelial cells can be seeded onto the top (or interior, if already tubular) of the lamina propria mesh, and smooth muscle cells can be seeded onto the bottom (or exterior, if already tubular) of the same mesh to form the muscularis mucosa.

In parallel, cells isolated from the muscularis externa can be seeded onto the surface of the submucosa, or the surface of the serosa, or both. Alternatively, the inner layer of the muscularis externa can be grown on the submucosal stromal mesh, and the outer layer can be grown on the serosal stromal mesh.

At appropriate stages of growth, these meshes can be combined using one of the methods outlined in Section 7.2. The cultures can be incubated until the surface layers are mature.

During the assembly of the different three-dimensional cultures, vascular tissue (i.e., arteries and veins) can be added to the tubular construct. For example, a blood vessel can be constructed in vitro as outlined in Section 8. Before combining the submucosal and serosal meshes, this blood vessel can be laid down longitudinally along one or both surfaces of the submucosal stromal culture. Upon implantation, it can be spliced to the appropriate blood vessel of the adjoining segment of the gastrointestinal tract.

By growing these layers separately, and then combining them and allowing further growth, distinct tissue layers can be formed and then allowed to mature in the same type of environment as naturally allows for their specialization.

In cases where only one of these layers has been damaged in the patient, a single three-dimensional culture would suffice, and can be implanted selectively to replace just that layer.

If a fully functional replacement with all the various layers of tissue is not required, a simple homogenous three-dimensional stromal culture lined with epithelium can be used. More layers of this homogenous stromal matrix can be combined to provide the appropriate thickness for such a prosthesis.

9. GENITOURINARY TRACT

Another embodiment of the invention provides for the replication of genitourinary tract elements in vitro in a

system comparable to physiological conditions. The genitourinary tract is very similar to the digestive tract in terms of histology. The primary differences can be the smaller diameters and lack of absorptive surface of the genitourinary vessels.

9.1. Ureter

Like the gastrointestinal tract, the ureter also has a mucous membrane as its inner layer. Despite not having an absorptive surface, the interior surface of the ureter is highly folded to form a stellate conformation in cross-section. The epithelial lining, however, is four to five cells thick. The lamina propria, which lies beneath the epithelium, contains abundant collagen, some elastin, and occasional lymph nodules.

Surrounding the mucous membrane is a muscular coat, whose inner layer contains longitudinally arranged smooth muscle fibers, while those of the outer layer are circularly arranged. The outermost layer, the adventitia, consists of fibroelastic connective tissue.

In order to construct a simulation of a ureter in accordance with the invention, stromal cells can be isolated from the two connective tissues associated with the ureter and used to initiate two separate three-dimensional cultures as described in Section 6.1. After appropriate growth of the stromal layers, epithelial cells can be seeded on the interior side of the lamina propria derived culture, and smooth muscle cells can be seeded onto the opposite surface. In parallel, smooth muscle cells can be seeded onto one surface of the adventitia derived culture. The two three-dimensional cultures can then be combined to form one tubular structure, as described in Section 6.2., and incubated until the surface layers are mature.

If a fully functional replacement with all the various layers of stromal tissue is not required, a simple homogenous three-dimensional stromal culture lined with epithelium can be used. More layers of this homogenous stromal matrix can be combined to provide the appropriate thickness for such a prosthesis.

9.2. Urethra

The urethra consists simply of a lamina propria which is lined with epithelium and surrounded by two layers of smooth muscle fibers. In the inner layer, the fibers are arranged longitudinally, while in the outer layer they are circular. The connective tissue of the lamina propria is rich in elastic fibers and contains many venules.

Since the urethra has only one stromal layer, a single three-dimensional culture may suffice for its construction in vitro in accordance with the invention. A mesh can be inoculated, for example, with cellular material isolated from the patient's urethral lamina propria as described in section 7.1. At the appropriate stage of confluence, epithelial cells can be seeded onto one surface (interior) and smooth muscle can be seeded onto the opposite surface (exterior). The three-dimensional culture can be incubated until the surface layers are mature.

10. HERNIA REPAIR

In herniorrhaphy, a corrective bioresorbable three-dimensional mesh, seeded with fibroblast cells could be used. Alternatively, cells might be seeded onto a synthetic mesh substrate for stronger fascial closure.

11. FORMATION OF TENDONS AND LIGAMENTS

Ligaments and tendons consist of fibroblasts surrounded by fibers of collagen type I and III and a predominance of the glycosaminoglycan dermatan sulfate. The embodiment of the invention provides for the placement of stromal tissue under mechanical or pulsatile forces to alter the formation and alignment of collagen fibers into bundles more dense and parallel than those routinely seen in dermis. By placing dermal fibroblasts on polymers and growing the tissues under increasing pulsing mechanical force, the final structure will have the tensile strength of a normal tendon (~33 MPa). Ligamentous or tendinous structures are also created utilizing similar methods with the option of attaching tissue-engineered bone to the end of the forming ligament or tendon in order to provide an attachment site.

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. A tubular living stromal tissue prepared in vitro, comprising stromal cells and connective tissue proteins naturally secreted by the stromal cells attached to and substantially enveloping a three-dimensional tubular framework composed of a biocompatible, non-living material having interstitial spaces bridged by the stromal cells.

2. The tubular living stromal tissue of claim **1** in which the stromal cells are fibroblasts.

3. The tubular living stromal tissue of claim **1** in which the stromal cells are human dermal fibroblasts.

4. The tubular living stromal tissue of claim **1** in which the stromal cells are fibroblasts, pericytes, macrophages, monocytes, plasma cells, mast cells, adipocytes, umbilical cord cells, bone marrow cells from umbilical cord blood, smooth muscle cells, mucosal cells from genitourinary tract, mucosal cells from the nasopharyngeal lining, endothelial cells or a combination thereof.

5. The tubular living stromal tissue of claim **1** in which the framework is composed of a biodegradable material.

6. The tubular living, stromal tissue of claim **5** in which the biodegradable material is polyglycolic acid, cotton, cat gut sutures, cellulose, gelatin, collagen or polyhydroxyalkanoates.

7. The tubular living stromal tissue of claim **1** in which the framework is composed of a non-biodegradable material.

8. The tubular living stromal tissue of claim **7** in which the non-biodegradable material is a polyamide, a polyester, a polystyrene, a polypropylene, a polyacrylate, a polyvinyl, a polycarbonate, a polytetrafluorethylene, or a nitrocellulose compound.

9. The tubular living stromal tissue of claim **1**, **2**, **3**, **4**, **5**, **6**, **7** or **8** in which the framework is a mesh.

10. A method for implantation of a tubular living stromal tissue, comprising implanting the tubular living stromal tissue of claim **1**.

11. A method for preparing a tubular living stromal tissue in vitro, comprising culturing stromal cells previously inoculated onto a three-dimensional tubular framework composed of a biocompatible, non-living material in a culture medium, so that the stromal cells and connective tissue proteins secreted by the stromal cells attach to and substantially envelope the framework to produce a three-dimensional tubular structure having interstitial spaces bridged by the stromal cells.

12. The method of claim 11 in which the stromal cells are fibroblasts.

13. The method of claim 11 in which the stromal cells are human dermal fibroblasts.

14. The method of claim 11 in which the stromal cells are fibroblasts, pericytes, macrophages, monocytes, plasma cells, mast cells, adipocytes, umbilical cord cells, bone marrow cells from umbilical cord blood, smooth muscle cells, mucosal cells from the gastrointestinal tract, mucosal cells from genitourinary tract, mucosal cells from the nasopharyngeal lining, endothelial cells or a combination thereof.

15. The method of claim 11 in which the framework is composed of a biodegradable material.

16. The method of claim 15 in which the biodegradable material is polyglycolic acid, cotton, cat gut sutures, cellulose, gelatin, collagen or polyhydroxyalkanoates.

17. The method of claim 11 in which the framework is composed of a non-biodegradable material.

18. The method of claim 17 in which the non-biodegradable material is a polyamide, a polyester, a polystyrene, a polypropylene, a polyacrylate, a polyvinyl, a polycarbonate, a polytetrafluorethylene, or a nitrocellulose compound.

19. The method of claim 11, 12, 13, 14, 15, 16, 17, or 18 in which the framework is a mesh.

20. The method of claim 11 further comprising culturing parenchymal cells inoculated onto the living stromal tissue.

21. The method of claim 20 in which the parenchymal cells comprise smooth muscle cells, endothelial cells, mucosal cells from gastrointestinal tract, mucosal cells from genitourinary tract, mucosal cells from the nasopharyngeal lining, or endothelial cells, fibroblasts, umbilical cord cells, or bone marrow cells from umbilical cord blood.

22. The method of claim 11 in which the culture medium is kept under static conditions.

23. The method of claim 11 in which the culture medium is kept in dynamic state by recirculating the culture medium.

24. A three-dimensional tubular blood vessel tissue, comprising endothelial cells cultured on a tubular living stromal tissue prepared in vitro, which stromal tissue comprises stromal cells and connective tissue proteins naturally secreted by the stromal cells attached to and substantially enveloping a three-dimensional tubular framework composed of a biocompatible, non-living material having interstitial spaces bridged by the stromal cells.

25. A three-dimensional tubular gastrointestinal epithelial tissue, comprising gastrointestinal mucosal epithelial cells cultured on a tubular living stromal tissue prepared in vitro, which stromal tissue comprises stromal cells and connective tissue proteins naturally secreted by the stromal cells attached to and substantially enveloping a three-dimensional tubular framework composed of a biocompatible, non-living material having interstitial spaces bridged by the stromal cells.

26. A three-dimensional tubular genitourinary epithelial tissue, comprising genitourinary epithelial cells cultured on a tubular living stromal tissue prepared in vitro, which stromal tissue comprises stromal cells and connective tissue proteins naturally secreted by the stromal cells attached to and substantially enveloping a three-dimensional tubular framework composed of a biocompatible, non-living material having interstitial spaces bridged by the stromal cells.

27. A method for preparing a tubular living stromal tissue in vitro, comprising culturing stromal cells previously inoculated onto a three-dimensional framework composed of a biocompatible, non-living material in a culture medium, so that the stromal cells and connective tissue proteins secreted by the stromal cells attach to and substantially envelope the framework to produce a three-dimensional structure having interstitial spaces bridged by the stromal cells; and rolling the three-dimensional structure into a tubular living stromal tissue.

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⑭発明の名称　生体組織用充填材

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明細書

1．発明の名称

生体組織用充填材

2．特許請求の範囲

1．コラーゲンスポンジと生体分解吸収性高分子材料との複合材料より成ることを特徴とする生体組織用充填材。

2．繊維状の生体分解吸収性高分子材料がコラコラーゲンスポンジ中に混在し、もしくは埋入されて成ることを特徴とする請求項（1）項記載の生体組織用充填材。

3．生体分解吸収性高分子材料がポリ－L－乳酸であることを特徴とする請求項（1）、（2）項記載の生体組織用充填材。

3．発明の詳細な説明

（産業上の利用分野）

本発明は、損傷、欠損等の外科的治療、整形外科手術等に使用される充填材に関する。

（従来技術）

損傷、欠損等の外科的治療、及び、整形外科手術等においては組織の再生、拘縮を防止する目的において、欠損部に充填材が埋入される。

かかる素材としては、組織反応が少なく、線維芽細胞の増殖を促し、組織が再生するまで長期にわたってその強度、形状が維持される機能が求められる。また、特に適用中においては組織の拘縮を防止する目的において保形性を有する機能が求められ、また、組織の再生後においては異物として体内に残留することなく速やかに消失することが理想とされる。

かかる目的に対し、ミクロポーラスなコラーゲンスポンジが提案されているが、上記の機能を満足しない。

（発明が解決しようとする問題点）

即ち、例えば、グルタールアルデヒドを用いて架橋させたコラーゲンスポンジは、生体に埋入後2～3ヶ月後には完全に生体に分解吸収されて消失してしまい治癒に必要な長期の強度、保形性を維持しない。

本発明は、かかる従来の欠点を解消し、組織反

応が少なく、且つ、線維芽細胞の増殖を促すと共に、長期にわたって形状、強度が維持され、また治癒後は生体に吸収される新規な充填材を提供したものである。

（問題を解決するための手段）

しかるに、本発明はコラーゲンスポンジと生体分解吸収性高分子材料との複合材料より成り、かかる生体分解吸収性高分子材料として繊維状のポリ－Ｌ－乳酸を用いたこと、およびこれをコラーゲンスポンジ中に混在、もしくは埋入させて構成したことに特徴を有するものである。

（作用）

本発明は、コラーゲンスポンジ中に生体内での分解速度の遅いポリ－Ｌ－乳酸を混在させて複合化させたことによってスポンジ構造のｐｏｒｅを長期にわたって維持でき、また、繊維状のポリ－Ｌ－乳酸との複合化によって内部への線維芽細胞の増殖を促すと共に、治癒に必要な長期にわたっての強度、形状の維持を可能としたものである。

以下、その構成について、例示する。

（構成例）

３デニールのポリ－Ｌ－乳酸糸（分子量80000）０．３ｇをからめてスライバー状とし、これを縦、横、深さが夫々６×２×２ｃｍの容器に入れ、これに豚由来のアテロコラーゲン0．３％塩酸溶液５０ｇを１８００ｒｐｍにて６０分間撹拌して注いだ。次いで、これを４８時間凍結乾燥し、アルコールにて滅菌して本発明充填材を構成した。

このようにして得た充填材は、ミクロポーラスなスポンジ構造の間にポリ－Ｌ－乳酸糸がランダムに埋入されて複合化された外観を呈した。

また、その物性値は第１表に示すように従来のコラーゲン単独のスポンジと比較し、破断強度、破断伸度、ヤング率が格段に高い値を示し、著しい改善が成された。また、PoreSizeも大きくなっている。

尚、表における比較例は架橋剤としてグルタールアルデヒドを使用した豚由来のアテロコラーゲン０．２％塩酸溶液５０ｇを前記と同様の方法によって処理して得たコラーゲン単独のスポンジで

ある。

第１表

	強　度	伸　度	ヤング率	PoreSize
本発明	7.7	132	27.8	97
比較例	1.3	48	8.0	63

尚、これの測定はＪＩＳ法に準じた。また、その単位は以下の通りである。

強度：破断強度（×10^6）、(dyne/cm²)

伸度：破断伸度（％）

ヤング率：（×10^6）、(dyne/cm²)

PoreSize：(μm)

上記の方法により得た本発明充填材を以下の方法により動物実験に供し、組織学的検討と肉細の状態を観察した。

（適用例）

体重３５０ｇのウィスター系ラットの背部筋層上を２×２ｃｍ大に剥離し、その部分に約２ｃｍ

大の本発明充填材を埋植し、経過を観察した。

＜１ヶ月後＞

スポンジ内周部分で線維芽細胞の侵入が認められるが中央部では細胞未侵入。

＜３ヶ月後＞

スポンジ中央部への細胞侵入は２ヶ月後に比べて増加している。

＜６ヶ月後＞

スポンジ中央部へ線維芽細胞が一定の方向で並ぶ部分ができた。

組織学的検討において埋植３～４ヶ月後にスポンジの中央部まで線維芽細胞が十分に侵入し、６ヶ月後においては完全に組織が構築された。

一方、拘縮の状態については石膏模型により、その容積を測定する方法によって行なったが、上記比較例によるものは２ヶ月後で初期体積の５～１５％しか残存せず、４ヶ月後では殆ど生体内に吸収され、消失するという結果であったが、本発明充填材によると６ヶ月後においても初期体積の３５～５０％が残存し、かかる面においても顕著

な差が見られた。

（発明の効果）

以上のように本発明による充填材は、その実用結果からも明らかなように、用途上の要求特性である、組織反応がないこと、線維芽細胞の増殖を促すこと、組織が再生するまで長期にわたってその強度、形状が維持されること、組織の陥縮を防止する機能を有すること、組織の再生後は体内に分解吸収されて消失してしまうこと等、この種の用途に必要な機能を全て兼ね備えたものであり、効果的な適用が可能なものである。

尚、コラーゲンスポンジと生体分解吸収性高分子材料との複合化比率、およびポリーL－乳酸繊維の繊度等はその用途、必要機能等に応じて任意に選択可能なものである。

以上のように本発明は、従来にない新規な構成の生体組織用充填材を提供したものである。

特許出願人　グンゼ株式会社
代表者　遠藤源太郎

MORITA ET AL., JP 3-23864

Specification

1. Title of the Invention

Filler material for living tissue

2. Claims

1. A filler material for living tissue, characterized in comprising a composite material of collagen sponge and a bioabsorbable polymer material.

2. A filler material for living tissue in accordance with Claim 1, characterized in that a fibrous bioabsorbable polymer material is mixed into or embedded in the collagen sponge.

3. A filler material for living tissue in accordance with Claim 1 or Claim 2, characterized in that the bioabsorbable polymer material is poly-L-lactic acid.

3. Detailed Description of the Invention

(Industrial Applicability)

The present invention relates to a filler material which may be employed in the surgical treatment of wounds and defects and the like or in orthopedic surgery.

(Background Art)

In the surgical treatment of wounds or defects or the like, and in orthopedic surgery, filler material is embedded in damaged areas in order to regenerate tissue and to prevent contracture.

It is required of such materials that they have little reactivity with tissue, that they promote the proliferation of fibroblasts, and that they maintain their strength and shape over a long period of time until the tissue is regenerated. Furthermore, it is a particularly required property that such materials maintain their shape in order to prevent contracture of the tissue

during actual use, and additionally, that they rapidly disappear from within the body and do not remain as a foreign object after the regeneration of the tissue.

Microporous collagen sponges have been proposed for such purposes; however, they do not have the above properties.

(Problem to be Solved by the Invention)

That is to say, collagen sponges in which, for example, glutaraldehyde is cross-linked do not maintain the requisite long-term shape and strength required for use in treatment, and within two to three months of three implantation in the body, they are completely broken down and absorbed by the body and disappear.

The present invention solves the defects present in the prior art; it provides a novel filler material having little reactivity with tissue and which promotes the propagation of fibroblasts, maintains its shape and strength over a long period of time, and furthermore is absorbed into the body after treatment.

(Means for Solving the Problem)

Moreover, the present invention is characterized in that it comprises a composite material consisting of collagen sponge and a biodegradable polymer material, fibrous poly-L-lactic acid is employed as the biodegradable polymer material, and this material is mixed into or embedded in the collagen sponge.

(Function)

By combining poly-L-lactic acid, which is slow to degrade within the body, with the collagen sponge, the present invention makes it possible to maintain the structural pores of the sponge over a long period of time, and furthermore, to promote the propagation of fibroblasts in

the interior of the material by means of the combination with fibrous poly-L-lactic acid, and also to maintain the strength and shape over the long period of time required for treatment.

Hereinbelow, the composition will be described.

(Embodiment)

0.3 g of 3-Denier poly-L-lactic acid fibers (molecular weight 80,000) were twined in a sliver, and placed in a vessel having length, width, and height dimensions of 6 x 2 x 2 cm, and this was agitated for a period of 60 minutes at 1,800 rpm with 50 g of a 0.3% hydrochloric acid solution of porcine atherocollagen. Next, this was freeze-dried for a period of 48 hours and sterilized in alcohol to produce the filler material of the present invention.

The filler material obtained in this manner had the appearance of a composite in which poly-L-lactic acid fibers were randomly embedded in a microporous sponge structure.

Furthermore, as shown in Table 1, in comparison with the prior art sponge composed only of collagen, the rupture strength, rupture ductility, and Young's modulus of the present invention are considerably higher, and it represents a dramatic improvement. Furthermore, the pore size is larger.

The comparative example in the Table is a sponge comprising only collagen that was prepared by a method identical to that described above using 50 g of a 0.2% hydrochloric acid solution of porcine atherocollagen, using glutaraldehyde as a crosslinking agent.

Table 1

	Strength	Ductility	Young's Modulus	Pore Size
Present invention	7.7	132	27.8	97
Comparative Example	1.3	40	8.0	63

These values were obtained by the JIS methods. Furthermore, the units are as given below.

Strength: rupture strength (x 10^5) (dyne/cm^2)

Ductility: rupture ductility (%)

Young's Modulus: (x 10^5) (dyne/cm^2)

Pore Size: (μm)

The filler material of the present invention obtained by the method described above was employed in animal testing using the following methods, and the histology, strength, and state of contracture thereof were assessed.

(Applied Example)

A 2 x 2 cm section of the back muscle of a 350 g Wistar rat was removed, and an approximately 2 cm section of the filler material of the present invention was implanted at this spot, and the progress thereof was observed.

(After One Month)

The infiltration of fibroblasts into the peripheral portions of the sponge was confirmed, but the cells had not infiltrated into the central portion thereof.

(After Three Months)

The cellular infiltration into the central section of the sponge was increased in comparison with after two months.

(After Six Months)

In portions of the central part of the sponge, the fibroblasts were arranged in a single direction.

Histologic studies revealed that fibroblasts had sufficiently penetrated the central part of the sponge three to four months after implantation, and the tissue was completely regenerated after six months.

The state of contracture was assessed using a method in which the volume was measured by means of plaster modeling. Using the comparative example above, only approximately 5-15% of the initial volume remained after two months, and after four months, the absorption into the body was complete, and the material had disappeared. In contrast, using the filler material of the present invention, 35-50% of the original volume was present, even after six months, and this represents a striking difference.

(Effects of the Invention)

As is clear from the effects obtained when the filler material of the present invention was applied, as described above, the material has the required properties for use and does not react with tissue, promotes the propagation of fibroblasts, maintains its strength and shape over a long period of time until the regeneration of the tissue, functions to prevent contracture of the tissue, and is broken down and absorbed into the body after the regeneration of tissue, so that the material has all the properties necessary for use, and may be effectively employed.

The proportions in which the collagen sponge and the bioabsorbable polymeric material are combined, as well as the size of the poly-L-lactic acid fibers and the like may be appropriately selected in accordance with the required properties.

As described above, the present invention provides a biodegradable filler material having a novel composition which was not conventionally available.

US005882929A

United States Patent [19]

Fofonoff et al.

[11] Patent Number: 5,882,929

[45] Date of Patent: Mar. 16, 1999

[54] **METHODS AND APPARATUS FOR THE CONDITIONING OF CARTILAGE REPLACEMENT TISSUE**

[75] Inventors: **Timothy W. Fofonoff**, Dedham; **Eugene Bell**, Boston, both of Mass.

[73] Assignee: **Tissue Engineering, Inc.**, Boston, Mass.

[21] Appl. No.: **56,675**

[22] Filed: **Apr. 7, 1998**

[51] **Int. Cl.**[6] **C07C 00/00**; C12M 3/00

[52] **U.S. Cl.** 435/395; 435/284.1; 435/286.5; 600/36; 73/790; 73/807; 73/818; 73/843; 73/846; 73/847

[58] **Field of Search** 435/1.1, 1.2, 395, 435/402, 283.1, 284.1, 286.5; 600/36; 606/57; 73/788, 790, 813, 814, 818, 841, 843, 846, 847, 807

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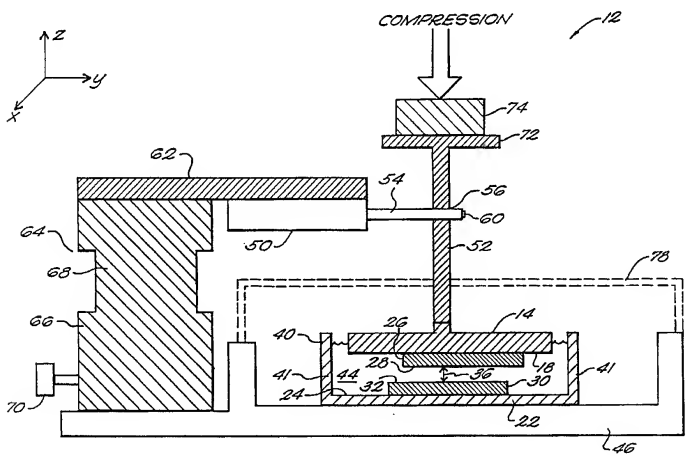
Primary Examiner—David A. Redding

Attorney, Agent, or Firm—Lahive & Cockfield, LLP; Anthony A. Laurentano

[57] **ABSTRACT**

Apparatus and methods are disclosed for maturing a biopolymer tissue construct in vitro prior to use as a replacement construct in vivo as, for example, a graft, implant, or prosthesis. The tissue is seeded with specific cells, exposed to a maturation fluid, such as a synovial-like fluid containing hyaluronic acid, and subjected to selected conditioning and maturation forces, which can include frictional forces, shear forces, and compressive pressure. The tissue is mounted on a first support element and a second surface applies a selected force to the tissue. This maturation process occurs within a maturation chamber. The resultant matured replacement tissue construct is intended to provide a replacement tissue that is more readily integrable in vivo to produce a more durable and functional replacement tissue.

86 Claims, 9 Drawing Sheets



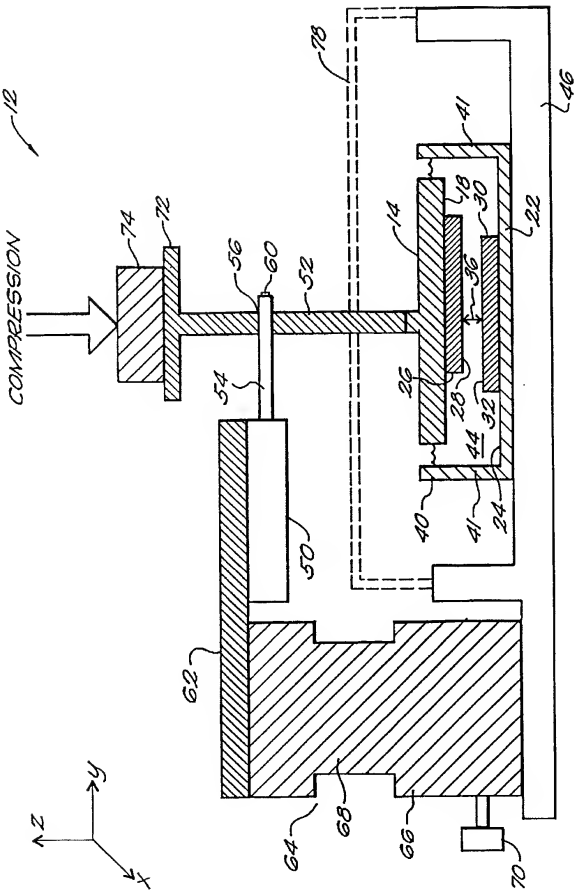

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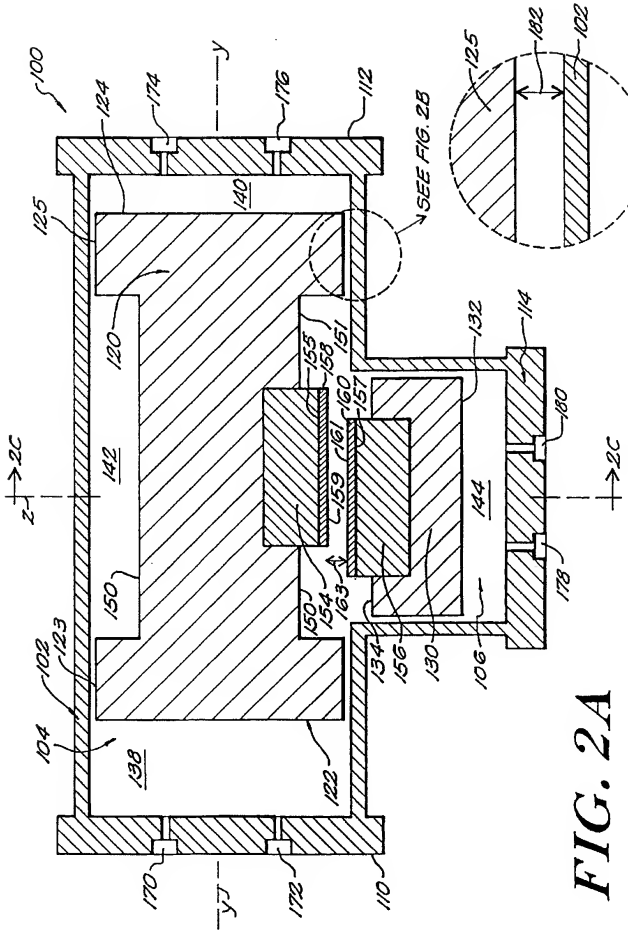
FIG. 1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y
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140
SEE FIG. 2B
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132
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157
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180
142
144
150
154
163
178
102
123
134
156
130
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138
122
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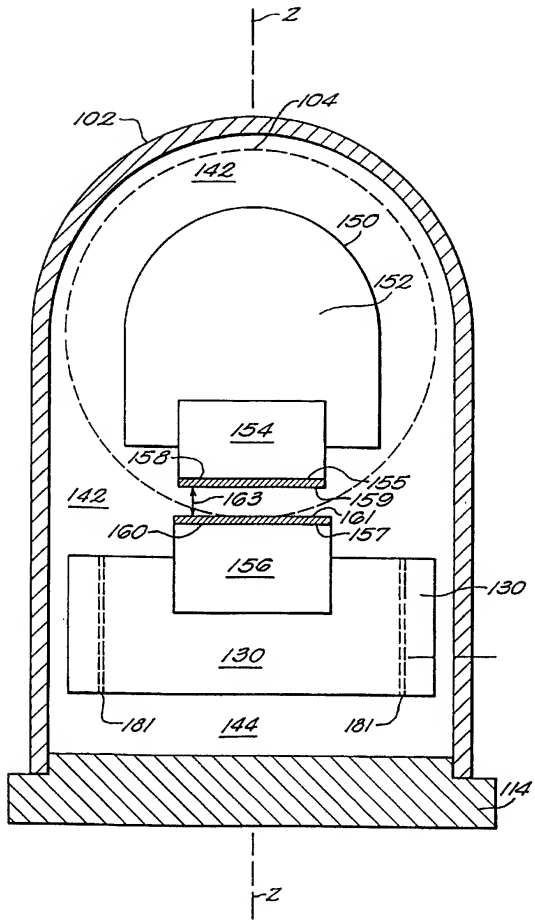
2C

FIG. 2A

125
182
102

FIG. 2B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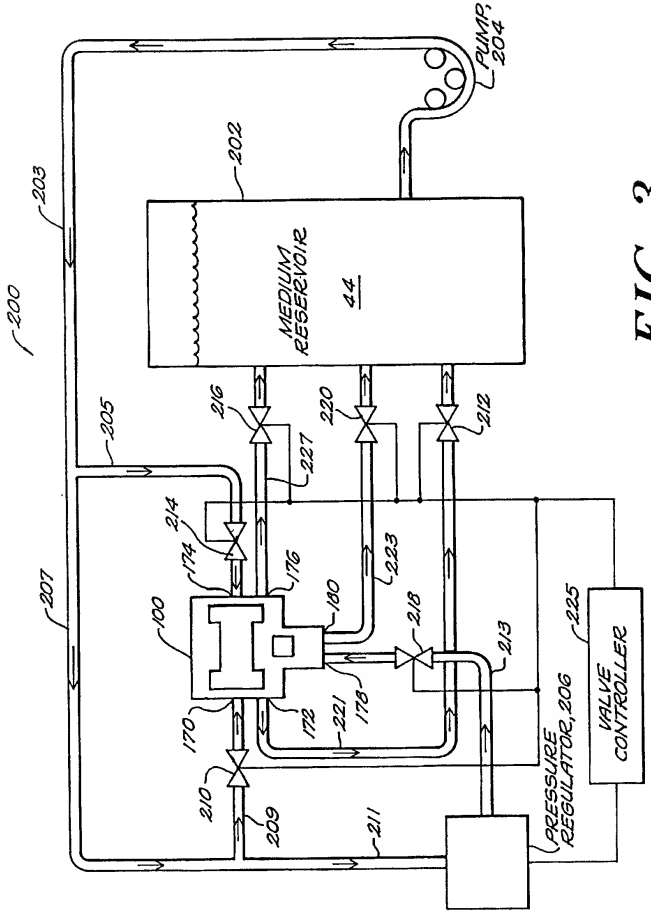
114
Z

FIG. 2C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PUMP, 204
MEDIUM RESERVOIR 44
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176
223
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213
225
170
172
221
178
VALVE CONTROLLER
210
209
211
PRESSURE REGULATOR, 206

FIG. 3

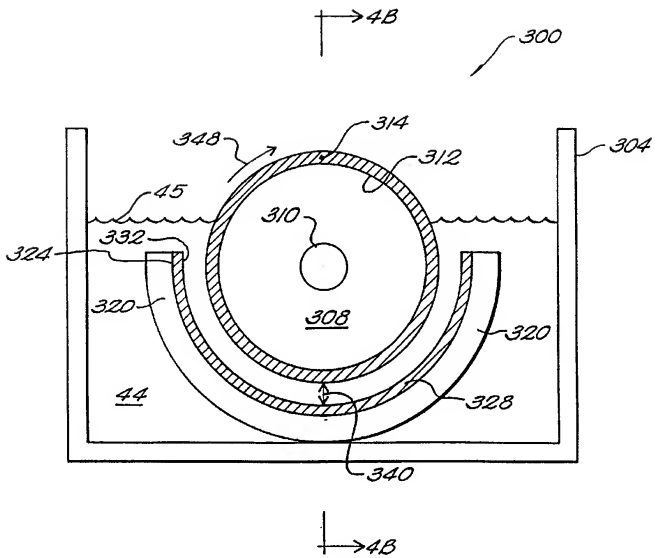
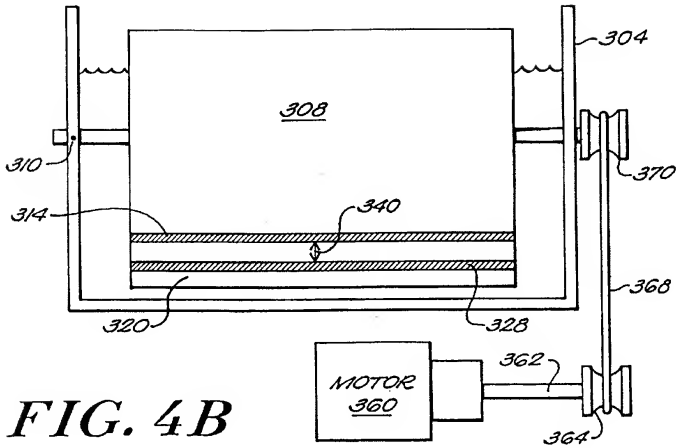


FIG. 4A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308
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MOTOR
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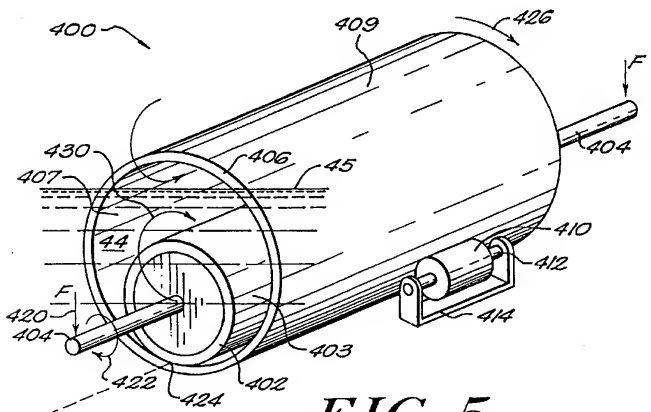
364

FIG. 4B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422
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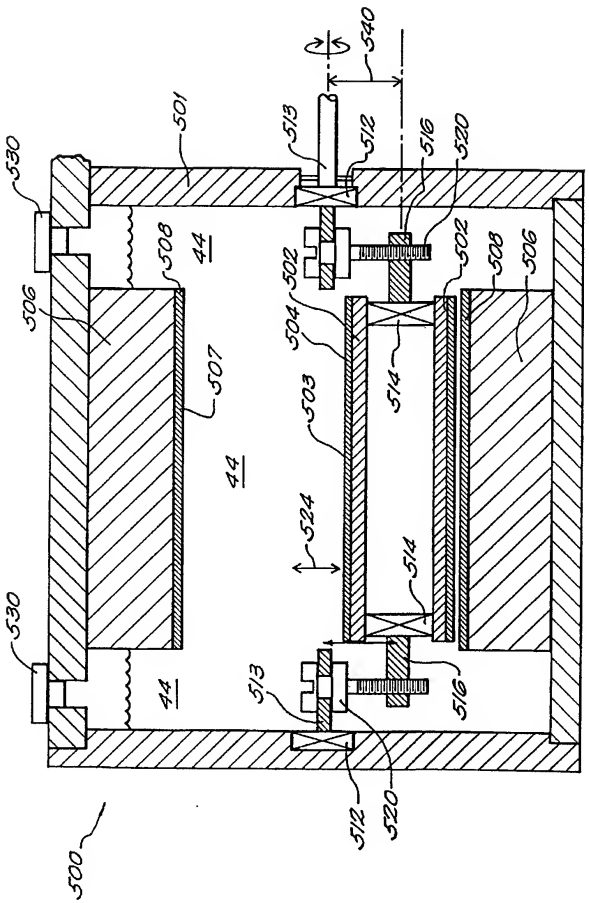
402

FIG. 5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540
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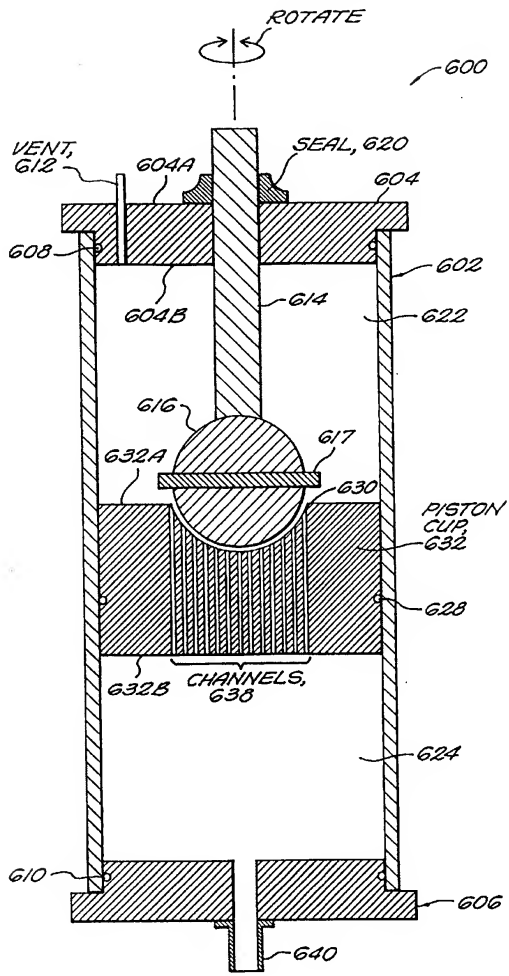
FIG. 6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VENT, 612
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CHANNELS, 638
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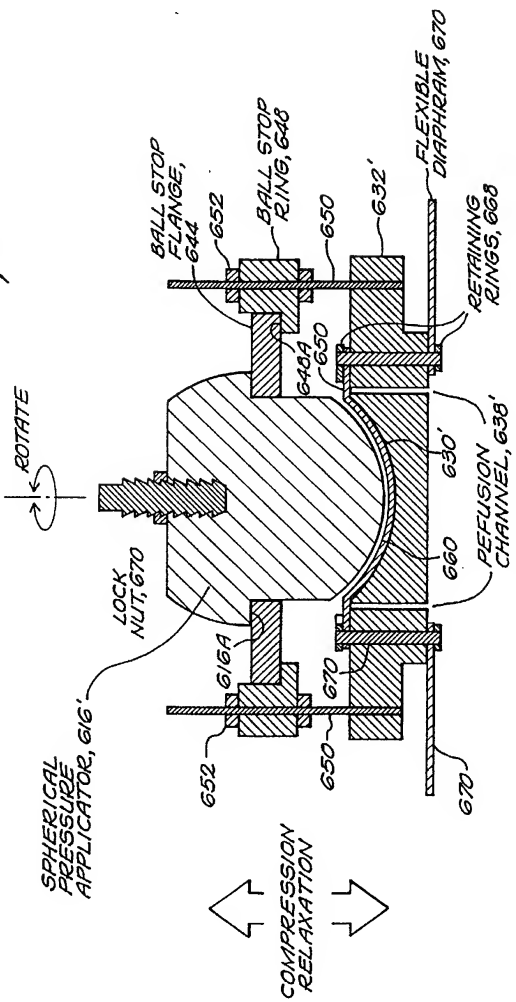
610
606
640

FIG. 7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ROTATE
BALL STOP FLANGE, 644
652
BALL STOP RING, 648
650
632'
FLEXIBLE DIAPHRAM, 670
RETAINING RINGS, 668
644A
650
630'
PEFUSION CHANNEL, 638'
660
LOCK NUT, 670
616A
670
SPHERICAL PRESSURE APPLICATOR, 616'
652
650
670
COMPRESSION
RELAXATION

FIG. 8

METHODS AND APPARATUS FOR THE CONDITIONING OF CARTILAGE REPLACEMENT TISSUE

BACKGROUND OF THE INVENTION

This application relates to the preparation of grafts, implants, prostheses or other tissue constructs, typically for use as replacements for damaged or diseased bodily tissue. More particularly, this application relates to the maturation, or conditioning, of biopolymer tissue constructs prior to implantation of the construct in the body.

Tissue constructs are often used as grafts, implants or prostheses to replace diseased or damaged bodily tissue. Tissue needing replacement can include, for example, cartilage, tendon and ligament tissue. A fully functional replacement tissue should withstand at least the stresses and strains imposed by normal bodily activity on the type of tissue the construct is to replace. Furthermore, the construct should be biocompatible and integrable, in vivo, i.e., the construct should resemble a natural tissue so as to attract and interact with specific cells present in the body. The attracted cells further organize the construct and secrete specific biosynthetic products, such as extracellular matrix proteins and/or growth factors, that bind to the replacement construct, enabling it to degrade, remodel and regenerate as a fully functional replacement tissue. Such integration strengthens and conditions the construct to better perform as a replacement tissue.

Synthetic materials such as polyester fibers (Dacron™) or polytetrafluoroethylene (PTFE) (Teflon™) have been used extensively as replacements for bodily tissue, with some success. However, due to the poor biocompatibility of such synthetic materials, they often initiate persistent inflammatory reactions. Additionally, they do not readily breakdown and are not readily integrated with the body via remodeling by tissue cells.

It is also known to fabricate replacement constructs from structural biopolymer matrix components, such as collagen, that are extracted, purified and combined with specialized cells. The cells can organize, condense, and otherwise interact with the matrix proteins to create a tissue-like construct that can more closely resemble a natural tissue, and hence more readily integrate with the body than implants, grafts or prostheses based on synthetic materials. However, available biopolymer implants do not always have, or develop in vivo, the matrix complexity characteristic of the tissue they are to replace so as to become fully-functional replacements.

Therefore, there is a need for improved replacement tissue constructs that are stronger and more readily integrable with the bodily environment.

Accordingly, one object of the invention is to provide methods and apparatus for producing improved replacement tissue constructs.

Another object of the invention is to provide replacement tissue constructs that are stronger and more capable of withstanding the stresses and strains imposed thereon by the rigors of bodily activity.

A further object of the invention is to provide prostheses, grafts and implants that are more readily accepted by and integrable with the natural bodily environment.

Another object of the invention is to provide prostheses, grafts and implants that more readily resemble the tissues they are intended to replace.

Other objects and advantages of the present invention will be appreciated by one of ordinarily skill in the art from the following disclosure, including the drawings and the claims.

The invention will next be described in connection with various preferred embodiments. However, it should be clear that various changes and modifications can be made by those skilled in the art without departing from the spirit and scope of the invention.

SUMMARY OF THE INVENTION

The present invention attains the foregoing and other objects by providing methods and apparatus for exposing biopolymer replacement tissue or tissue constructs to selected conditions, i.e., to a selected maturation fluid and to selected forces and/or stresses, for maturing the replacement tissue prior to insertion of the tissue into the body.

As used herein, maturing a tissue refers to conditioning a tissue such that it is more integrable with the bodily environment. Hence, an integrable tissue is a tissue that more readily remodels, degrades and regenerates, within the body, to create a stronger and more durable functional replacement tissue.

As used herein, a biopolymer is a polymer suitable for introduction into a living organism, e.g., a human. The biopolymer is usually non-toxic and bioabsorbable when introduced into the living organism, and any degradation products of the biopolymer are also non-toxic to the organism. The biopolymer can be formed into biocompatible constructs that include, for example, biopolymer foams, e.g., single or double density foams, and/or biopolymer fibers. A typical biopolymer is collagen.

Typically, biopolymer tissue to be matured using the apparatus and methods of the present invention is seeded with cells, such as chondrocyte cells obtained from a biopsy of human or animal tissue. Exposing the replacement tissue to maturation fluid and subjecting the tissue to selected forces is considered to provide an environment that, similar to the natural bodily environment, provides biological signals to the seed cells for producing an integrable replacement tissue more readily accepted by the body. For example, the biological signals provided by the methods and apparatus of the present invention may promote, in addition to other beneficial effects, the secretion of extracellular matrix material, the generation of cell binding sites that attract specific cells from the body and/or cell differentiation.

A useful maturation fluid is a thixotropic, synovial-like fluid that transmits shear forces to the biopolymer tissue as a result of relative motion between the tissue surface and a second surface that faces the tissue surface, and which is spaced therefrom. The selected forces and/or stresses imposed upon the tissue can include shear forces, frictional forces, torsional forces and compressive forces. The invention is intended to be particularly useful for the maturation of replacement cartilage tissue.

According to one aspect, the invention provides an apparatus that includes a first support element having a first surface for receiving and mounting a biopolymer tissue and a second support element having a second surface facing a surface of the biopolymer tissue. The first and second surfaces can be spaced apart to form a gap. Also included are a fluid element, such as reservoir, for introducing a maturation fluid to the tissue received by the first surface and to the second surface, and a relative motion element coupled to one or both of the first and second support elements for providing relative motion between the first and second surfaces. The relative motion subjects the tissue to selected forces, such as shear forces transmitted by the maturation fluid, or direct frictional forces due to contact of the first tissue surface and the second surface of the second support

element. The fluid element typically confines the maturation fluid and maintains the fluid in communication with the biopolymer tissue received and mounted by the first surface, such as by immersing the two surfaces in the maturation fluid and filling any gap therebetween with fluid. The fluid element, however, need not be a reservoir that immerses the surfaces in a volume of confined fluid. The fluid element can include nozzles that direct flow of maturation fluid towards the biopolymer tissue and the second surface.

According to another aspect of the invention, the second support element is adapted for receiving and mounting a second biopolymer tissue such that the second support surface contacts the second biopolymer tissue.

According to another feature of the invention, the second surface of the second support element is spaced from the tissue along a first axis, and the relative motion element translates the tissue in a plane substantially transverse to the first axis. The relative motion element hence linearly reciprocates the first support element relative to the second support element.

The relative motion element typically translates the biopolymer tissue received and mounted by the first surface of the first support element relative to the second surface of the second support element at speeds ranging between about 0.5 cm/sec and about 50 cm/sec.

According to yet a further feature, the invention includes an element for varying the spacing, i.e., the gap, between the first and second tissue surfaces. The gap can typically be varied between about 0 mm (where direct contact occurs) and about 5 mm. In one aspect, the gap can be eliminated such the tissue surface contacts the second support surface and the relative motion element slidingly and frictionally engages the tissue surface and the second surface.

According to yet another aspect, the invention includes a compression element for subjecting at least the tissue to selected compressive pressure. The compression element can be adapted for generating a compressive pressure on tissue of between about 0 psi and about 100 psi. The compression element can compress the biopolymer tissue mounted on the first surface of the first support element against the second support element, thereby subjecting the tissue to a compressive force. The compressive force can be applied in the absence of relative motion between the first tissue surface and the second surface, or with relative motion therebetween, such that the surfaces slidingly engage and frictional forces as well as compressive pressure are exerted on the tissue surface.

The maturation fluid is typically a fluid that resembles the naturally occurring synovial fluid. For example, the maturation fluid can be a dialysate of blood plasma, or an imitation thereof, that contains hydraulic acid. For maturing a replacement cartilage tissue, the tissue is seeded with chondrocyte cells that can be obtained from tissue samples that include cells from bone marrow or differentiated articular cartilage. Typically, cells are dissociated enzymatically with collagenase and cultivated in vitro to expand upon the primary cell populations. For seeding a biopolymer replacement tissue formed of a biopolymer foam typical cell suspensions provide between about 5×10^4 and about 1×10^6 cells per milliliter of foam.

Many variations of the present invention are possible. According to one embodiment, an apparatus according to the invention includes a fluid reservoir for holding a maturation fluid, a first support element, such as a block, adapted for mounting a first sheet of biopolymer tissue, and a second support element, such as another block, adapted for mounting a second sheet of biopolymer tissue spaced, typically along a vertical axis, from the first biopolymer tissue to form a substantially uniform gap therebetween. The first and second support elements are positioned relative to the reservoir so as to immerse at least a portion of the first and second biopolymer tissues in the maturation fluid, thereby introducing the maturation fluid to the gap so as to contact the first and second biopolymer tissues.

A translation element can translate the first support element relative to the second support element so as to translate the first tissue relative to the second tissue. The translation element can be an electromechanical actuator, coupled to the first support element, for linearly reciprocating the first support element, typically along an axis transverse to the vertical axis.

According to another aspect, the apparatus further includes a compression element coupled to at least one of the first and second support elements for pressing together the first and second biopolymer tissues. The compression element can include a platform, coupled to one of the support elements, for receiving a weight. The gravitational force on the weight is transferred to one of the support elements for pressing the support elements together.

In yet a further aspect, a variable spacing element, such as translation stage, can be included for varying the spacing between the first and second biopolymer tissues.

In another embodiment, the invention includes a rotatable inner cylinder support element having an outer circumferential surface adapted for mounting a first biopolymer tissue such that the mounted biopolymer tissue has a first outwardly-facing tissue surface. An extended arcuate outer support element, such as a hemi-cylinder, is spaced from the inner cylinder and has an inner mounting surface adapted for mounting a second biopolymer tissue having an inwardly facing tissue surface. The rotatable inner cylinder and the outer hemi-cylinder are spaced apart such that the outwardly facing tissue surface is spaced from the inwardly facing tissue surface to form a gap therebetween, and the rotatable inner cylinder and the outer hemi-cylinder are operatively arranged with a fluid reservoir such that the maturation fluid held therein is introduced to the gap and contacts at least a portion of the inwardly and outwardly facing tissue surfaces. The apparatus further includes a rotating element, such as an electric motor, for rotating the rotatable inner support cylinder. Typically, the rotatable inner cylinder and the hemi cylinder are mounted coaxially.

In one aspect of this embodiment, the gap between the outwardly and inwardly facing tissue surfaces can vary. In another aspect of the invention, the outwardly facing tissue surface is spaced from the inwardly facing tissue surface by between about 0 mm and about 5 mm. The apparatus can further include a compression element for compressing together the inwardly and outwardly facing tissue surfaces. In another aspect, the tissue surfaces can be slidingly engaged, with or without compressive pressure, and the tissue surfaces can be compressed together, with or without relative motion therebetween.

In yet another embodiment, an apparatus according to the invention includes a housing having first and second bores formed therein, the first bore having first and second ends and extending along a first longitudinal axis, and the second bore having first and second ends and extending along a second longitudinal axis disposed at an angle, typically transverse, to the longitudinal axis of the first bore. The second end of the second bore intersects and fluidly communicates with the first bore between the first and second ends of the first bore.

An extended piston is disposed for travel in the first bore, and has an extended piston first face, an extended piston second face, and an interconnecting section extending between the first and second faces. Adjacent the first and second piston faces are longitudinally extending first and second piston skirts, respectively, that are separated by a selected gap from the wall of the first bore. An outer surface of the interconnecting section is adapted for receiving and mounting a first biopolymer tissue. A transverse piston is disposed for travel in the second bore, and has a transverse piston first face and a transverse piston second face, the second face being disposed for receiving and mounting a second biopolymer tissue facing the first biopolymer tissue. Typically, the transverse piston includes first and second transverse piston skirts that are adjacent the first and second transverse piston faces, respectively, and are separated by a selected gap from the wall of the second bore.

The extended piston divides the first bore into a first volume, bounded in part by the extended piston first face and the first end of the first bore, and a second volume, bounded in part by the extended piston second piston face and the second end of the first bore. The first and second bores intersect and define a third volume bounded in part by the outer surface of the interconnecting section of the extended piston and the transverse piston second face. A fourth volume is bounded in part by the first end of the second bore and the first face of the transverse piston.

Fluid ports in the bores can allow the transfer of a first fluid, such as maturation fluid, from a fluid supply element, or system, to selected volumes, such as to the first, second and fourth volumes. A fluid port can be included for direct transfer of fluid to the third volume. Alternatively, fluid can be transferred to and from the third volume from the first, second or fourth volumes in various manners, such as an orifice in one of the piston faces, or a suitable gap between the skirt of a piston face and the wall of the housing.

A fluid supply system can selectively transfer fluid to and from, and modulate the pressure of fluid in, the above volumes for providing relative motion between the tissues, for varying the gap between the tissues, for exerting a compressive force on the tissues, and for slidingly engaging the tissues with or without a compressive force. The fluid supply apparatus can include a fluid reservoir, a fluid pump, a pressure regulator, and appropriate valves and conduit for controlling fluid flow to the various fluid ports of the above embodiment. For example, selectively transferring fluid to and from the first and second volumes can selectively translate the extended piston in the first bore.

According to yet another embodiment, apparatus according to the invention resembles a "rolling pin," and has an inner cylinder having a first radius of curvature disposed within the lumen of an outer cylinder. The inner and outer cylinders extend along parallel first and second central axes, respectively. The first central axis can be offset, along a line transverse to the central axes, from the second axis.

The inner cylinder has an outer surface for receiving a first biopolymer tissue having an outwardly facing tissue surface. The outer cylinder includes a wall, having an inner face, bounding the lumen, and for receiving a second biopolymer tissue having an inwardly facing tissue surface. The outwardly facing tissue surface of the first biopolymer tissue mounted on the inner cylinder faces the inwardly facing tissue surface of the second biopolymer tissue mounted on the outer cylinder.

In one aspect of the invention, the inwardly facing tissue surface is spaced from the outwardly facing tissue surface by a gap. The apparatus can include, as in other embodiments, a spacing element for varying the gap, such as an element for moving the inner cylinder relative to the outer cylinder in a direction transverse to the central axes. For example, the inner cylinder can be mounted on a shaft, and the position of the shaft can be varied for varying the gap between the tissue surfaces. The gap between the tissue surfaces can be non-uniform, i.e., the gap can vary around the circumference of the cylinders. For example, the spacing element can move the inner cylinder shaft such that the inner cylinder central axis is offset from the outer surface central axis.

According to another feature, the invention includes an element for introducing a maturation fluid into the gap for contacting the outwardly and inwardly facing tissue surfaces. For example, the inner and outer cylinders can be arranged with a fluid reservoir such that the maturation fluid enters and at least partially fills the lumen of the outer cylinder.

According to one aspect, the invention includes an element for rotating the inner cylinder and/or the outer cylinder. For example, the element can be a motor coupled to the shaft of the inner cylinder. The motor can be operative with the spacing element such that the gap can varied. For example the motor and shaft can be supported by a fixture that is moved by the spacing element.

According to another feature, the outwardly facing tissue engages, i.e., contacts, the inwardly facing tissue along a engagement line substantially parallel with the central axes. For example, the spacing element can adjust the offset of the cylinder central axes such that the tissue surfaces engage along the engagement line. In another aspect of the invention, one of the inner or outer cylinders can be coupled to a rotational drive element, such as a motor, for rotating the cylinder. The other cylinder can be fixed, such that the tissues slidingly engage at the engagement line, or the other cylinder can be rotationally mounted, such as with bearings or other rotational support element, so that the cylinder being rotated by the rotational drive element rotates the other cylinder. The outwardly facing and inwardly facing tissues thus frictionally engage at least along the engagement linc, but do not frictionally and slidingly engage.

According to yet another feature of the invention, a compression element presses the outwardly facing tissue and the inwardly facing tissue together to exert sclected compressive force on the outwardly and inwardly facing tissue surfaces, typically along the engagement line.

In a further feature of the invention, an element is included for translating the inner cylinder and outer cylinders relative to each other to vary the offset between the central axes thereof such that the line of engagement is circumferentially varied about the inwardly facing tissue surface. For example, the inner cylinder can be mounted on a shaft that is coaxial with the central axis of the inner cylinder. The shaft can be rotationally mounted by a bearings on each end of the shaft to a mounting fixture. The mounting fixture can be translated in an arcuate, typically circular, path in a plane transverse to the central axes of the inner and outer cylinders, such that the line of engagement varies circumferentially about the inwardly facing tissue. The inner cylinder can rotate, due to the contact along the line of engagement between the inwardly facing and outwardly facing tissues, as the inner cylinder is translated within the lumen of the outer cylinder. The outwardly and inwardly facing tissue surfaces thus frictionally engage, but do not frictionally and slidingly engage.

Translation of the cylinders relative to each other may be combined with at least one rotational drive element, such as a motor, for rotationally driving at least one of the cylinders.

The above embodiments involve attaching a biopolymer tissue to a portion of the apparatus, such as a support element, which can include a block, a portion of a piston, or a surface of a cylinder. A biopolymer tissue can be a foam, and can be attached by a variety of means, such as bone cement, to a support element, or can be cast as a foam directly on a support element. Casting of foams is discussed below. It is believed that attaching a tissue with bone cement may have certain advantages. The maturation process may promote intergrowth of the biopolymer tissue and the bone cement, and a portion of the bone cement can be implanted in the body with the biopolymer tissue. The bone cement attached to the tissue can be cemented to bone existing in the body.

Forces need not be applied to the replacement tissue throughout the maturation process, nor need the composition of the maturation fluid remain constant. For example, for expansion of the seed cell population, the maturation fluid can contain cell nutrients. During expansion of the chondrocyte seed population, forces are typically not applied to the replacement tissue. However, to provide biosignals to promote cell differentiation and/or secretion of the extracellular matrix material, forces are typically applied and cell nutrients are of lesser importance as a component of the maturation fluid, than for example, growth factors for promoting proper cell differentiation. Thus the apparatus and methods of the present invention are intended to provide a versatile tissue-maturation tool that one of ordinary skill in the art, based on the disclosures herein, can use to tailor the conditioning of a replacement tissue. According to the invention, tissue can be matured not only for implantation but as part of a research study, in which case the exact program of forces and maturation fluid composition could be varied to determined the effect on tissue development. Research efforts may result in a improved or optimized program that is then applied to the maturation of tissue constructs for usc in vivo.

The invention also includes methods practiced in accordance with the teachings of the invention presented herein.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, features and advantages of the invention will be apparent from the following description and apparent from the accompanying drawings, in which like reference characters refer to the same parts throughout the different views. The drawings illustrate principles of the invention and, although not to scale, show relative dimensions.

FIG. **1** is a cross-sectional view of one embodiment of an apparatus for maturing biopolymer replacement tissue prior to implantation of the tissue into the body according to the teachings of the present invention.

FIGS. **2**A through 2C illustrate cross-sectional views of another embodiment of an apparatus according to the invention for maturing biopolymer replacement tissue prior to implantation of the tissue into a body. FIG. 2B is an exploded view of the vessel employed in FIG. 2A. FIG. 2C is a cross-sectional view, taken along section line C—C in FIG. 2A, of the apparatus illustrated in FIG. 2A.

FIG. **3** is a schematic system flow diagram of a maturation fluid supply system for supplying fluid to, and for actuating, the tissue maturation apparatus of FIGS. 2A through 2C.

FIGS. 4A through 4B are cross-sectional views of still another embodiment of an apparatus for maturing replacement tissue prior to implantation of the tissue into a body. FIG. 4B is a cross-sectional view taken along section line B—B of FIG. 4A illustrating a motor for powering the apparatus shown in FIGS. 4A and 4B.

FIG. **5** is a perspective view of yet another embodiment of an apparatus according to the invention for maturing tissue prior to insertion of the tissue in a body.

FIG. **6** is a cross-sectional view of one embodiment of an apparatus for maturing tissue in accordance with the features illustrated in FIG. 5.

FIG. **7** is a plan view of a double-curvature embodiment of the tissue maturation apparatus according to the teachings of the present invention.

FIG. **8** is a partial cross-sectional view of an alternate embodiment of the spherical applicator of FIG. 7 according to the teachings of the present invention.

DESCRIPTION OF THE ILLUSTRATED EMBODIMENTS

FIG. **1** shows an apparatus **12** for maturing biopolymer replacement cartilage tissue prior to implantation of the cartilage tissue in, for example, a knee joint. The illustrated tissue maturation apparatus **12** includes a first support element **14** having a first outer surfacc **18** and is adapted for receiving and mounting biopolymer tissuc **26**. A second support element **22** having an outer surface **24** is spaced along the Z axis, as shown in FIG. **1**, from the first support element **14**. The first outer surface **18** mounts a first sheet of biopolymer tissue **26**. A layer of bone cement (not shown) can be interposed between the first sheet of tissue **26** and the outer surface **18** to attach the sheet of tissue **26** to the surface **18**. A contact surface **28** of thc biopolymcr tissue **26** is spaced by a substantially uniform gap **36** from a second outer surface **32** of a second sheet of biopolymer tissue **30** mounted to the second support element **22**. The second sheet of biopolymer tissue **32** is optional, and if not present, the gap **36** separates the contact surface **28** of the first biopolymer tissue **26** from the outer surface **24** of the second support element **22**.

A reservoir **40** confines a maturation fluid **44** therein such that the maturation fluid **14** substantially fills the gap **36** and is in communication with at least a portion of each of the biopolymer tissue surfaces **28** and **32**. The second support element **22** and sides **41** form the reservoir **40**. The apparatus base **46** supports the reservoir **40** as well as other components of the tissue maturation apparatus **12**, as described below.

A vertical rod **52** and a horizontal actuating rod **54** couple the first support element **14** to a relative motion element **50**. The horizontal actuating rod **54** can have a bore **56** formed therethrough, through which the vertical rod **52** passes. Vertical rod **52** can be affixcd to the actuating rod **54** via a set screw **60** disposed axially in the actuating rod **54**. The relative motion element **50** can be a linear actuator motor which reciprocates the actuating rod **54**, and hence the first support element **14** and the first cartilage tissue **26** attached thereto, along the Y axis.

The maturation fluid **44** in the gap **36**, in response to the linear reciprocating motion of the first cartilage tissue **26**, generates a shear force that acts on the first surface **28** of the first tissue sheet **26**, and, if the second tissue sheet **30** is present, on the second tissue surface **32**. The magnitude of the shear force gcnerated by the apparatus **12** is a function of the nature of the maturation fluid, the size of the gap **36**, and the stroke and frequency of the linear reciprocating motion of the first tissue imposed by the linear actuator **50**.

The support plate **62** supports the linear actuator **50** and couples the actuator **50** to a variable spacing element **64**. The

variable spacing element **64** includes a lower fixed portion **66** and an upper translation stage **68**. Rotation of knob **70** translates the upper stage **68** relative to the fixed portion **66** along the vertical or Z axis, thereby varying the gap **36** between first biopolymer tissue surface **28** and the second biopolymer tissue surface **32**, or if the second biopolymer tissue **30** is omitted, between the first biopolymer tissue surface **28** and the surface **24**.

The upper end of the vertical rod **52** includes a platform **72** adapted for receiving a weight **74**. Loosening the set screw **60** allows the vertical rod **52** to move downwards until the first surface **28** of the first biopolymer tissue **26** contacts the second surface **32** of the second biopolymer tissue **30**. Various weights can be placed on the platform **72** for generating a selected pressure between the surfaces **28** and **32** of the biopolymer tissues **26** and **30**. The actuating means **50** can provide a relative motion as the pressure duc to weight **74** is applied to the biopolymer tissue surfaces **28** and **32**. Optionally a cover **78** can be employed to cover the reservoir **40**, first mounting element **14** and second mounting element **22**, as well as biopolymer tissues **26** and **32** and the maturation fluid **44**.

The cartilage articulation apparatus **12** is an open system, i.e., exposed to the surrounding environment, and is typically disposed in an incubator (not shown) that maintains a 10% CO_2 atmosphere. The 10% CO_2 atmosphere of the incubator communicates with the maturation fluid or culture medium in the reservoir **44** to maintain the pH of the maturation fluid **44** in a selected range. A suitable linear actuator **50**, having a stroke of 1 inch, is available from Menzinuer Aircraft Components, Inc. of Vista, Calif. as part number MAC-S4-102. The MAC-S4-102 operates on low voltage direct current, and the polarity of the voltage can be periodically reversed so as to reciprocate at approximately 2 strokes per minute, via switching gear known to those of ordinary skill in the art. The support element **14** and the second support element **42** can be fabricated from polymeric or TEFLON blocks or other suitable biocompatible, non-leaching material. The cartilage tissues **26** and **30** can be affixed thereto with calcium phosphate bone cement. A suitable vertical translation stage **64** is available as part number D39928 from the Edmund Scientific Company of Barrington, N.J.

FIGS. 2A, 2B, and 2C illustrate an alternate embodiment of a tissue maturation apparatus **100** according to the present invention. FIG. 2B is an enlargement of a portion of FIG. 2A, and FIG. 2C is a sectional view of apparatus **100** taken along section line C—C.

The tissue maturation apparatus **100** shown in FIGS. 2A through 2C is a closed system, i.e., it is not in communication with the ambient atmosphere. The tissue maturation system **100** includes a housing **102** having two bores formed therein. The first bore **104** extends along the axis Y—Y and is intersected by a transverse bore **106** that extends along the axis Z—Z. The first bore is bounded by a first bore end **110** and a second bore end **112**, and in part by a first transverse bore end **114**. An extended piston **120** is disposed for travel in the first bore **104** and includes a first extended piston face **122** and a second extended piston face **124**. A transverse piston **130** is disposed for travel in the transverse bore **106** and has a first transverse piston face **132** and a second transverse piston face **134**. The extended piston **120** divides the first bore **104** into a first volume **138** bounded along the Y axis by said first extended piston face **122** and the first bore end **110**, and into a bore volume **140** bounded along the Y axis by the second extended piston face **124** and the second bore end **112**. Similarly, the transverse piston **130** creates a third volume **142** bounded in part by the second transverse piston face **134** and the outer surface **150** of the interconnecting section **152** of the extended piston **120**. The transverse piston **130** also divides the transverse bore into a fourth volume **144** bounded along the Z axis by the first transverse bore end **114** and the first transverse piston face **132**.

A portion of the outer surface **150** of the interconnecting section **152** of the extended piston **120** is adapted so as to form a first mounting elemcnt **154** to mount a first biopolymer tissue construct. Likewise, the transverse piston face **134** is apertured to form a second mounting element **156** for mounting a second biopolymer tissue construct. Specifically, the first surface **155** of the first mounting element **154** mounts a first sheet of biopolymer tissue **158**, typically spaced across a substantially uniform gap **163** from a second sheet of biopolymer tissue **160** mounted by a second face **157** of the second mounting element **156**.

Fluid ports **170** and **172**, formed in the first end **120** of the housing **102** communicate a maturation fluid **44** (not shown) with the first volume **138**. Similarly, fluid ports **174** and **176** in the second end **112** of the housing **102** communicate the maturation fluid **44** with the bore volume **140**. Fluid ports **178** and **180** in the first end **114** of the transverse bore **106** allow communication of maturation fluid **44** with transverse bore volume **144**. The maturation fluid **44** is selectively supplied or drawn through fluid ports **170**, **172**, **174** and **176**, as required, to reciprocatingly translate the extended piston **120** within the bore **104** along the Y axis. Selectively supplying or drawing the maturation fluid **44** from fluid ports **178** or **180**, as required, varies or adjusts the gap **163** and the compressive forces between the biopolymer tissue **158** and the biopolymer tissue **160**. Thus, provision of a maturation fluid to fluid ports **170**, **172** or **174**, **176** allows for reciprocating motion of the piston and hence of the first biopolymer tissue mounted thereto. The reciprocating motion moves the first tissue relative to the second tissue.

The frictional and compressive forces applied to the tissues **154**, **156** can be adjusted or varied according to a selected program of compressional loading and unloading by a control system (not shown). The controller can be in open or closed feedback connection with the apparatus to vary or to maintain the frictional and/or compressional forces applied to the tissues. Those of ordinary skill will readily be able to determine in light of the present teachings the type and magnitude of the forces that the tissue is subjected to, based upon the intended site of the tissue, the intended use, the type of maturation fluid and seeded cells, and the type and size of the apparatus.

A sufficient amount of the maturation fluid **44** (not shown) should be present in the gap **163** so as to contact a portion of the first biopolymer tissue surface **159** and a portion of the second biopolymer tissue surface **161** such that relative motion of the tissue surfaces creates shear forces thereon. The maturation fluid **44** can enter the bore volume **142** between the ends **123**, **125** by, for example, passing through a gap **182** formed about the outer diameter of the piston and between the extended piston and the walls of the housing **102**, as illustrated in FIG. 2C. The maturation fluid then flows between bore volumes **138**, **140**, and **142**, such that it can contact the surfaces **159** and **161** of tissues **158** and **160**, respectfully. The gap **182** is not so large as to prevent a reasonable pressure differential to form easily and consistently between the bore volumes **140** and **138** to translate the extended piston **120** in the bore **104** to provide relative, reciprocating motion between the biopolymer tissue surfaces **159** and **161**. Such a gap **182** is not the only means by

which the maturation fluid **44** can be supplied to the gap **163** so as to contact the biopolymer tissue surfaces **159** and **161**. For example, an orifice **181**, FIG. 2C, of a selected diameter may be provided through transverse piston **130**, or an appropriate gap (not shown) provided between the skirt of piston **130** and the walls of housing **102**, to provide a flow of maturation fluid **144** from the bore volume **144** to the bore volume **142**. Such an orifice can be used in conjunction with gap **182** to allow maturation fluid to flow from the bore volume **144** to the volume **142**, and then to the bore volumes **138** and **140**. Alternatively, a fluid port (not shown) can be added to the housing **102** to communicate maturation fluid directly with bore volume **142**.

FIG. **3** illustrates a fluid supply system **200** for supplying maturation fluid to the tissue maturation chamber **100**. The illustrated system **200** provides a selected flow of maturation fluid to selected bore volumes for reciprocatingly translating the extended piston **120** in the bore **104** and for translating the transverse piston **130** in the transverse bore **106** to adjust the gap **163**. In addition, the system **200**, in conjunction with the tissue maturation chamber **100**, provides a flow of maturation fluid to the gap **163** so as to immerse at least a portion of the tissue surfaces **159** and **161** in maturation fluid. The fluid supply system **200** can also provide a selected pressure of maturation fluid in the volume **144**, and hence to piston **130** for applying selected pressure between tissue surfaces **159** and **161**.

The fluid supply system **200** depicted in FIG. **3** includes a maturation fluid reservoir **202** containing maturation fluid **44**, a pump **204**, a pressure regulator **206**, a valve controller **225** and valves **210**, **212**, **214**, **216**, **218** and **220**, all connected as shown. In particular, the reservoir **202** is connected by fluid conduits **203**, **205**, **207** and **209** to the maturation chamber **100** through valves **210** and **214**. The valves **210** and **214** selectively control the introduction of fluid to the maturation chamber, as indicated by the flow arrows. Fluid conduit **211** connects the conduit **207** to the pressure regulator **206**. The pressure regulator output is connected by fluid conduit **213** to the maturation chamber **100** through valve **218**. The maturation chamber dispels maturation fluid through selected output conduits, for example, through output fluid conduits **221**, **223** and **227**, which connect at the other end to the reservoir **202** through valves **212**, **220**, and **216**, respectively.

The illustrated valve controller **225** is in feedback circuit with the pressure regulator **206** and valves **210**, **212**, **214** and **216** to modulate selectively the flow of the maturation fluid **44** to fluid ports **170**, **172**, **174** and **176**, respectively, for reciprocatingly translating the extended piston **120** in the bore **104**. For example, opening valve **210** and closing valve **212** allows the maturation fluid **44** to flow, via port **170**, into volume **138** and to increase the pressure of the maturation fluid **44** therein. This displaces the extended piston **120** to the right in FIG. **2A**. Closing valve **214** and opening valve **216** allows the maturation fluid **44** to flow through fluid port **176** from the bore volume **140**, thus releasing maturation fluid pressure therein. Hence the extended piston **120** translates to the right in FIG. **2A**. One of ordinary skill in the art, based on the disclosures herein, will readily appreciate how selective control of the valves shown in FIG. **3** reciprocatingly translate the piston **120**, translate the transverse piston **156** for selecting a pre-determined gap size **163**, and apply a selected pressure to the transverse piston **130** for providing a selected pressure on tissue surfaces **159** and **161**.

Similarly, operating the valves **218** and **216** allows the spacing between the tissues **158** and **160** to be varied or pressure to be applied between the tissues **158** and **160**. For example, opening the valve **218** to admit a selected amount of fluid and then closing both valves **218** and **220** maintains a selected volume of fluid in the bore volume **144** such that a selected gap **163** may be maintained between the tissues **160** and **158**. If necessary, the valve **218** can remain open and the pressure regulator **206** can maintain a nominal pressure volume to compensate for fluid leakage therefrom to bore volume **142**. To apply a selected compressive force, i.e., pressure between the tissues **158** and **160**, valve **218** can be opened and the pressure regulator **206** operates to adjust the fluid pressure to maintain an elevated fluid pressure within volume **144**, thereby forming a selected pressure between tissues **158** and **160**.

FIGS. **4A** and **4B** show another embodiment **300** of an apparatus for maturing biopolymer replacement tissue. An inner cylinder **308** mounts on an outer surface **312** thereof a first biopolymer tissue **314** having an outwardly facing tissue surface **316**. A stationary outer arcuate and extended support element such as the hemi-cylinder **320** mounts on an inner surface **324** thereof a second biopolymer tissue **328**. The tissue **328** includes an inwardly facing tissue surface **332**. A shaft **310** mounts the cylinder **308** such that there is a selected gap **340** between the tissue surfaces **316** and **332**. A reservoir **304** confines a maturation fluid, the upper level of which is indicated by the wavy line **45**, such that the maturation fluid **44** fills the gap **340** and communicates with tissue **314** and **328**, and with tissue surfaces **316** and **332**.

FIG. **4B** shows a motor **360** having a rotatable shaft **362** and a pulley **364**. The pulley is coupled by means of a belt **368** to a pulley **370** mounted on the shaft **310**. The shaft **310** is rotatably coupled, such as by bearings and fluid seals (not shown) to the reservoir **304**. The motor **360** rotates the cylinder **308**, as indicated by arrow **348** in FIG. **4A**, for providing relative motion of the cylinder **308** and the transmission of shear forces between tissue surfaces **316** and **332**.

Typically, the motor **360** rotates the cylinder **308** such that the first biopolymer tissue surface **316** is translated at speeds ranging from about **0.5** cm per second to about **50** cm per second.

One of ordinary skill in the art, based on the teachings herein, can readily employ further additional appropriate elements for varying the gap **340** and for applying a compressive or frictional force on the tissue surfaces **332** and **316**.

FIG. **5** schematically illustrates the principle features of another embodiment of a tissue maturation apparatus **400** in accordance with the teachings of the present invention. To facilitate the discussion of certain illustrated features, miscellaneous parts of the apparatus are omitted for clarity, but which have been previously described herein. The illustrated apparatus **400** is similar to that shown in FIGS. **4A** and **4B** except that the hemi-cylinder **320** is replaced by a full cylinder **406**. The inner cylinder **402** can have a radius of curvature significantly different than that of the outer cylinder **406**. As shown in FIG. **5**, the shaft **404** mounts an inner cylinder **402**, which in turn mounts on its outer surface a first biopolymer replacement tissue having an outwardly facing tissue surface **403**. An outer cylinder **406** mounts on its inner surface a second biopolymer tissue having an inwardly facing tissue surface **407**. The inner cylinder **402** is disposed within a central lumen of the cylinder **406** such that the outwardly facing tissue surface **403** faces the inwardly facing tissue surface **407**. Rollers, such as the roller **410** mounted on a shaft **412** and rotatably coupled to a roller mount **414**, rotatably support the outer cylinder **406** by

contacting the outer surface **409** of the outer cylinder **406**. The outer cylinder can thus rotate as indicated by arrow **426**. The shaft **404** can be rotatably mounted by a bearing fixed to a frame and can be driven by a motor similar to the arrangement depicted in FIG. **4**B.

The outwardly facing tissue surface **403** contacts the inwardly facing tissue surface **407** along the engagement line **424**. Rotation of the shaft **404** in the direction indicated by reference numeral **422** causes the outer cylinder **406** to rotate in the direction indicated by arrow **426** due to frictional contact between the two tissue surfaces **403** and **407** along the engagement line **424**. A fluid reservoir can confine a maturation fluid such that it contacts the tissue surfaces **403** and **407** at least at the line of engagement **424** therebetween. The inwardly facing tissue surface **407** and the outwardly facing tissue surface **403** frictionally engage along the engagement line **424** but do not slidingly engage. Application of a force **420** downward on shaft **404** subjects the tissue surfaces **407** and **403** to a compressive force.

A second motor can be added to the drive shaft **412** such that the outer cylinder **406** can be rotated independently of the inner cylinder **402**. Thus the inwardly facing tissue surface **407** and outwardly facing tissue surface **403** can slidingly engage along line **424**. One of ordinary skill in the art will recognize that means can be included with the apparatus **400** shown in FIG. **5** for raising or lowering shaft **404** for maintaining a selected gap between the tissues surfaces **407** and **403** along the engagement line **424**. The apparatus **400** disclosed in FIG. **5** does not maintain a substantially uniform gap between the tissue surfaces **403** and **407**.

The use of the term "gap" above in connection with the illustrated embodiment indicates that the tissue surfaces do not contact. If a gap exists between the tissue surfaces **407** and **403**, the phrase "line of engagement" is intended to designate a line of minimum spacing between the inwardly and outwardly facing tissue surfaces, **407** and **403**, respectively.

FIG. **6** illustrates an apparatus for translating the shaft **404** in FIG. **5** along path **430** such that the line of engagement **424** between the inwardly facing tissue surface **407** and the outwardly facing tissue surface **403** is circumferentially varied about the inwardly facing tissue surface **407**. The illustrated tissue maturation apparatus **500** includes a housing **501** for confining the maturation fluid **44**. The drive shaft **513**, offset screws **520**, and offset shafts **516** mount an inner cylinder **502** within an outer cylinder **506**. The outer surface of the inner cylinder **502** receiving a first biopolymer tissue **504** having an outwardly facing tissue surface **503**. The inner surface of cylinder **506** receiving a second biopolymer tissue **508** having an inwardly facing tissue surface **507** facing the tissue surface **503**. Bearings **512** rotatably mount the drive shaft **513** to the housing **501**. The drive shaft **513** can be driven by a motor and pulley arrangement such as the motor and pulley arrangement disclosed in FIGS. **4**A and **4**B.

The bearings **514** mount the inner cylinder **502** to offset shafts **516**. Offset screws **520** are rotatably mounted and captured by the drive shafts **513** and threadingly engage the offset shafts **516** such that the offset **540** distance between the offset shafts **516** and the drive shafts **513** may be varied by rotating the screws **520**. Thus the path **430** in FIG. **5** followed by the offset shafts **516** can be varied such that the outwardly facing tissue surface **503** is spaced by a selected gap from the inwardly facing tissue surface **507**. Alternatively, the offset distance between the drive shaft **513** and offset shafts **516** may be selected such that the line of engagement **424** between the inwardly facing tissue surface **403** and the outwardly facing tissue surface **407** is circumferentially varied about the inwardly facing tissue surface **407**. Circumferentially refers to a circular path, in a plane transverse to the central axes, around the inwardly facing tissue surface **407**. The inner cylinder **502** thus rotates about the bearings **514** due to frictional contact along the line of engagement **424** between the tissue surfaces **403** and **407**. The offset screws **520** can be adjusted such that a selected pressure exists on each of the tissue surfaces **503** and **507**.

The housing **501** includes media access plugs **530** for filling the interior of the housing with maturation fluid **44** and for adjusting the offset screws **520** for varying the offset distance **510** between the offset shafts **516** and the drive shafts **513**. Should the distance indicated by reference numeral **540** be selected such that the tissue surface **503** does not contact the tissue surface **507**, means can be added to the apparatus **500** shown in FIG. **6** to independently rotate the inner cylinder **502** as the drive shaft **513** is rotated. Rotating the inner cylinder **502** thus insures that the portion of the tissue surface **503** that is closest to the tissue surface **507** is continuously varied.

Although the embodiments disclosed above typically mount two biopolymer tissues having each having a tissue surface facing, across a gap, a tissue surface of the other tissue, two separate tissues are not considered necessary to condition tissue. A single biopolymer tissue can be conditioned by. mounting it so as to face a non-tissue surface across an appropriate gap.

Sheets or other shapes of biopolymer tissue for maturation in accordance with apparatus and methods of the present invention can be received and mounted on mounting elements, or other appropriate mounting surfaces, with bone cement, as indicated above. However, biopolymer tissues for maturation can be cast as a biopolymer foam directly on mounting surfaces, and further processed, if desired, before maturation of the tissue in a maturation apparatus. Accordingly, the deposition and processing of biopolymer foams is discussed below.

FIG. **7** illustrates another embodiment of a tissue maturation apparatus **600** according to the teachings of the present invention. The illustrated apparatus **600** includes a housing **602** that mounts a pair of endcaps **604** and **606**. The endcap **604** mounts an O-ring **608** in an annular channel to form a fluid-tight seal between an inner wall of the housing **602** and the endcap. Likewise, the endcap **606** mounts an O-ring **610** in an annular channel to form a fluid-tight seal between an inner wall of the housing **602** and the endcap. The endcap **604** is apertured with a vent through passage **612** that extends between top **604**A and bottom **604**B surfaces of the endcap. The vent passage vents air from the housing chamber to enable a fluid to flow therein. The endcap **604** further includes a central aperture that mounts a shaft that terminates at one end with a spherical pressure applicator **616**. The spherical pressure applicator **616** can further include a foam clamp **617** disposed about the applicator. A seal **620** mounts to the top **604**A of the endcap to prevent fluid leakage from an inner chamber of the housing to the external environment. The illustrated endcap **606** has a port **640** formed therein sized for introducing a fluid from a fluid source to the chamber **624**, or for drawing fluid from the chamber.

A piston cup **632** is mounted within the housing chamber and divides the chamber into an input chamber **622** and an output chamber **624**. The piston cup **632** has an outer

diameter equal to or slightly smaller than the inner diameter of the housing, and preferably seats an O-ring **628** to prevent fluid passage along the outer diameter of the piston cup. Those of ordinary skill will recognize that the piston cup outer surface can be spaced from the inner wall of the housing to allow passage of fluid therealong.

The illustrated piston cup **632** has a concave surface feature **630** formed on the top surface **632A**. The concave surface feature **630** has a radius of curvature that closely matches the radius of curvature of the spherical support **616**. The input chamber is fluidly coupled to the output chamber **624** by a plurality of perfusion channels **638** that extend from the concave surface feature **630** to the bottom surface **632B** of the piston cup **632**. The perfusion channels allow a selected degree of maturation fluid introduced to the input chamber **622** to pass or diffuse therethrough and hence between the chambers **622** and **624**. The piston cup can be supported in the illustrated position by known means, including rigid fasteners.

The position of the spherical pressure applicator **616** can be varied to define a gap of selected width between the applicator and the concave surface feature **630**. For example, the spherical pressure applicator **616** can be raised or lowered to widen or reduce the gap size. The gap size defines among other things the type of force applied to a tissue sheet supported on the top surface **632A** of the piston cup **632**. For example, the spherical pressure applicator **616** can be positioned so as to contact the tissue to apply a compressive force to the tissue. The spherical pressure applicator **616** can then be rotated whilst in this position to concomitantly apply a frictional force to the tissue. According to another aspect, the spherical pressure applicator **616** can be spaced from tissue by a selected degree. The maturation fluid can flow across the tissue or the spherical pressure applicator **616** can be rotated to create a shear force on the tissue. The curvature of the applicator and the piston cup define a double curvature tissue maturation arrangement that is suitable for conditioning the tissue in the presence of a maturation fluid to particular stresses. The tissue conditioned in this manner is suitable for use as replacement knee cartilage, since the double curvature arrangement mimics the articulation stresses experienced by the joint in its natural environment.

In operation, maturation fluid is introduced to the input chamber **622** and the spherical pressure applicator **616** is positioned to apply a selected shear or compressive force. The spherical pressure applicator **616** can be rotated if desired to further apply a frictional force to the surface of the tissue. During the stressing of the tissue, the maturation fluid coats the tissue and perfuses through the channels **638** into the output chamber **624**, where the maturation fluid is removed through the port **640**.

FIG. **8** is a perspective close-up view of an alternate embodiment **700** of the spherical pressure applicator **616'** and piston cup **632'** of FIG. **7**. The apparatus is thus intended to mount within the vessel **602** of FIG. **7**. Like parts are designated with like reference numerals plus a superscript prime. The spherical pressure applicator **616'** has a shoulder portion **616A** that abuts an annular flange **644**. The flange is preferably affixed to the applicator element **616'**. The flange in turn is sized to seat on an annular shoulder portion **648A** of an annular stop ring **648**. Specifically, the outermost diameter of the shoulder portion **616A** is slightly greater than the outermost diameter of the stop flange **644** to enable vertical movement of the flange into and out of engagement with the stop ring **648**. The spherical pressure applicator **616'** has a lock nut **678** coupled to a top end portion to fixedly couple the applicator **616'** to the shaft **614** of the apparatus.

The illustrated stop ring **648** has a central aperture that seats a plurality of guide rails **650** and locking seal elements **652**. The stop ring **648** slidingly engages and moves about the guide rails **650** in a known manner. The seal elements **652** are further designed to lock the stop ring in place at a specified location. The location of the stop ring can define the downward most position of the pressure applicator **616'** relative to the piston cup **632'**. A tissue sheet **660** can be affixed to the top surface of the cup **632'** such that it conforms with the contours of the concave surface feature **630'**. The position of the stop ring **648** can be varied to adjust the gap size formed between the outermost region of the pressure applicator **616'** and the concave surface feature **630'**. The position of the these two elements relative to each other thus determines the type of force applied to the tissue **660**. For example, the stop ring **648** can be positioned to form a gap between the applicator **616'** and the tissue **660** to form shear forces when the fluid flows across the surface or when the applicator **616'** is rotated. According to another practice, the stop ring can be positioned to enable the applicator to contact the tissue to apply a selected compressive force thereto. If the applicator **616'** is rotated, it can also apply a frictional force to the tissue in the manner described in connection with FIG. **7**.

The illustrated piston cup **632'** includes a main body that is apertured with bores that mount one end of the guide rails **650**. As shown, the guide rails **650** are mounted to both the piston cup **632'** and the stop ring **648** to enable the stop ring to move relative to the piston cup. The tissue sheet **660** is secured to the top surface of the piston cup, and hence to the concave surface feature **630'** by a pair of retaining ring assemblies. The assemblies include a pair of retaining rings **668** that are coupled together by a plurality of posts **670** mounted within corresponding apertures formed in the piston cup. The retaining rings and posts thus serve to hold the tissue sheet in place during the maturation process. A plurality of perfusion channels **638'** are formed within the piston cup **632'** and extend between the input and output chambers **622**, **624**.

A flexible annular diaphragm **670** is attached to the piston cup **632'** by the retaining rings **668**. The diaphragm extends between the piston cup and the inner wall of the housing **602**, and is preferably attached to the housing to prevent fluid from leaking between the input and output chambers at locations other than the perfusion channels **638'**.

In operation, the stop rings **648** are placed at selected locations to determine the separation or gap between the applicator **616'** and the tissue **660**. The maturation fluid **44** is then introduced to the input chamber **622** of the housing **602**. The maturation fluid fills the input chamber and coats the tissue surface. The flexible diaphragm **670** prevents the maturation fluid from leaking to the output chamber around the piston cup **632'**. The spherical pressure applicator **616** is placed at a selected position, the downward most position of which is defined by the stop ring location, to apply a selected shear or compression force to the tissue **660**. For example, the applicator **616'** can be vertically positioned within the housing **602** at any selected location. The applicator in particular can be lowered until the flange **644** abuts the shoulder **648A** of the stop ring. The stop ring thus prevents the applicator from exceeding this downward most position. If the applicator is separated from the tissue by a predetermined amount, the applicator **616'** can be rotated to create a shear force on the tissue **660**. The tissue can undergo this maturation for a selected period of time and according to a predetermined treatment regimen. Those of ordinary skill will be readily able to determine the tissue maturation

regimen in light of the type of tissue sheet, the type of maturation fluid, the type of any seeding cells, the expected replacement site, and other factors.

The spherical pressure applicator **616'** can also be employed to apply a frictional force to the tissue sheet. For example, the stop ring **648** can be placed at a position that allows the applicator **616'** to contact the surface of the tissue **660**. The applicator in this position can apply a compressive of a selected degree based upon the amount of force applied normal to the tissue surface. The applicator **616'** is then rotated to apply a frictional force to the entire tissue corresponding to the radius of curvature of the applicator **616'** as it contacts the tissue mounted within the concave surface feature **630'**, which itself has a defined radius of curvature.

During tissue maturation, the maturation fluid coats the tissue and perfuses through the channels **638'** into the output chamber, where the maturation fluid is removed through the housing port, FIG. **7**.

The tissue construct employed in connection with the above-described tissue constructs can be either biopolymer fibers, biopolymer foams, or biopolymer mats. A more complete description of biopolymer tissue constructs suitable for use with the present invention is described below.

Fabrication of Biopolymer Foams

Many biopolymers can be formed into biocompatible foams, e.g., single or double density foams, composite foams, and biocompatible constructs which include biopolymer fibers, e.g., collagen fibers, biopolymer fabrics, e.g., collagen fabrics, and/or extracellular matrix particulate. As used herein, the term "foam" refers to a network of communicating microcompartments having biopolymer molecules and/or biopolymer filaments interspersed within the walls of the microcompartments. The biopolymer foams can be single density or double density foams. Double density foams have microcompartments that are smaller in volume, typically by a factor of approximately two to ten, than single density foams.

Examples of biopolymers which can be used to form a foam include collagen, alginic acid, polyvinyl alcohol, proteins, such as chondroitin sulfate, elastin, laminin, heparin sulfate, fibronectin and fibrinogen. A combination or mixture of one or more biopolymers can be used to form the biopolymer foams, and composite foams, i.e., combined single and double density foams, or combination of foams of diffcrent biopolymers, that can form the biopolymer constructs of the invention. For example, a combination of chondroitin sulfate and fibronectin can be used to form biopolymer fibers that can be incorporated with a biopolymer foam to form a biopolymer construct described herein. A preferred biopolymer is collagen.

The biopolymers foams, e.g., single or double density foams, can be formed into structures of any form or shape, e.g., strips, sheets, tubes, etc. Structures comprising biopolymer foams combined with polymer mesh, e.g., a Teflon mesh are possible. Biopolymer foams can be used with tissue culture inserts for multiple plates which can be used as molds in which foams and biopolymer constructs of the invention can be formed for cell culture. Polymer meshes used with the foams and foam compositions of the invention can expose cells contained on and within the foams and foam compositions to the atmosphere as, for example, when the foams and foam compositions are used as skin equivalents to stimulate formation of a stratum corneum. Both the meshes and culture inserts have the advantage of providing a means for handling the foams and foam compositions without requiring actual contact with the foams or foam compositions. The shaped structures into which the foams and foam compositions are made can mimic those of tissues or body parts to be replaced and thus can be used as prostheses or grafts which tissue cells remodel to promote regeneration of a replacement tissue in the recipient. Extracellular matrix particulates and/or viable cells can also be added to the biopolymers to further promote cell in growth and tissue development and organization within the foams.

Biopolymer foams can be produced by forming a biopolymer solution, freeze-drying the solution to form a biopolymer foam, and crosslinking the biopolymer foam. Alternatively, the foam can be formed by performing the crosslinking step prior to the freeze-drying step. The step of freeze-drying converts the biopolymer solution into a foam, i.e., a network of communicating microcompartments with biopolymer molecules and/or filaments interspersed throughout its walls. When the foam is crosslinked, it becomes physically stable and insoluble in aqueous solutions. Preferably, the biopolymer solution is polymerized, prior to freeze-drying, to form a biopolymer lattice. As used herein, a biopolymer lattice refers to a network of biopolymer filaments in which fluid is trapped. Biopolymer filaments are nanometer-sized forms of polymerized biopolymer molecules. For example, if the biopolymer is collagen, the collagen polymerizes into nanometer sized filaments by a process of self-assembly.

The biopolymer solution can be formed by treating the biopolymer in such a manner that it becomes soluble, e.g., by manipulating its pH to put it into solution, can be polymerized using methods of polymerization known in the art. For example, the biopolymer, e.g., collagen, can be polymerized to form a biopolymer lattice by manipulation of the pH of the biopolymer solution, e.g., by exposure to ammonium vapor or by adding base. As the pH of the solution rcaches neutrality, the biopolymer polymerizes. The rate of polymerization is proportional to temperature and can be controlled by regulating the temperature of the collagen solution.

After the biopolymer has been polymerized to form a biopolymer lattice, it is typically freeze-dried and/or crosslinked. Typically, the order of the these steps depends on the method of crosslinking used. For example, if the crosslinking method is a liquid phase method, e.g., the use of aldehydic crosslinking methods, the crosslinking step is performed prior to the freeze-drying step. Alternatively, if the crosslinking method is a solid phase method, e.g., use of ultraviolet radiation, the crosslinking step is performed after the freeze-drying step. Crosslinking of the biopolymer can be accomplished by use of crosslinking methods known in the art. For example, the biopolymer can be crosslinked by subjection to ultraviolet radiation or by treatment with chemical crosslinking agents such as carbodiimide, glutaraldehyde, acetaldehyde, formaldehyde, and ribose. The biopolymer can also be crosslinked by dehydrothermal crosslinking.

If desired, prior to freeze-drying, selected reinforcing material can be added to the biopolymer solutions. Such reinforcing materials include biopolymer fibers, threads, e.g., woven or braided threads, and/or fabrics, e.g., nonwoven fabrics, prepared, for example, by textile methods. Biopolymer threads, e.g., collagen threads, can be prepared by extruding the biopolymer in solution into a coagulation bath and transferring the biopolymer to a bath containing ethanol or acetone or another dehydrating solution. Alternatively, the thread can be dehydrated by subjection to vacuum-drying. The biopolymer thread can then be

crosslinked by, for example, methods described herein. An example of an apparatus for spinning and processing a biopolymer fiber, e.g., collagen fiber, is described in U.S. Ser. No. 08/333,414, filed Nov. 2, 1994, the contents of which are incorporated herein by references in their entirety. The threads can then be dried, spooled, for example, by pulling the moving thread over more rollers, stretching and drying it and then winding it onto spools. The threads can be woven or braided into fabric or other complex forms or constructs for use as described herein.

The term biopolymer fabrics is intended to include nonwoven biopolymer fabrics, are typically composed of a mat of entangled biopolymer fibers of a selected size and density. Typically, the nonwoven biopolymer fabrics are produced by spooling dry biopolymer fiber onto a drum of circumference equal to that of the length of an individual fiber element. Spooling is continued until the number of wraps of fiber on the drum equals the number of pieces of fiber required for the fabric. A cut is then made across the wound fiber in a direction parallel to the drum axis and the fibers are removed from the drum. A textile machine, such as a staple length cutter can be used to cut the fibers to a selected length. The fiber can then be crosslinked if it has not been previously crosslinked. The fiber is then dispersed in a volume of a phosphate buffer solution for a period of time to decrease its pH and soften the fiber. The fiber is transferred to a volume of water and agitated mechanically to produce entanglement of the fiber strands. The entangled fiber strands are sieved from the water onto a collection screen until they coat the screen in a mat of uniform density. The mat is then dried on the screen or dried after transfer to another surface, screen, or cell culture device. If desired, the nonwoven mat can be cut or punched into smaller shapes after drying.

The biopolymer solution can then be freeze-dried to form a foam. The freezing step can be a controlled freezing step performed according to the method described in U.S. Pat. No. 4,531,373, the contents of which are incorporated herein by reference. The freeze-drying cycle typically includes freezing, evacuation, and drying phases. The freezing temperatures suitable for formation of the biopolymer foams of the invention depend upon the concentration of the biopolymer in solution or in the biopolymer lattice. Thus, for a collagen lattice in which the collagen is at a concentration of about 5 mg/ml the freezing temperature is typically less than −26° C. The collagen lattice is exposed to this temperature for a period of about 1 hour. A vacuum is then applied to the collagen lattice as the temperature is slowly raised.

To prevent the formation of fissures in the foam and thus to allow for greater foam size, an anti-freeze polypeptide (AFP) or an anti-freeze glycoprotein (AFGP) can be added to the biopolymer solution prior to or during the freezing step. Examples of AFPs include the AFPs which belong to the AFP Types I, II, and III. For a detailed description of the different types of AFPs, see, e.g., U.S. Pat. No. 5,358,931, PCT publication WO 92/12722, and PCT publication WO 91/10361, the contents of which are incorporated herein by reference. These polypeptides and glycoproteins prevent the formation of large ice crystals during freezing of the biopolymer solution and also prevent the formation of crystals of recrystallization during the drying process. Large ice crystals can create fissures in the resulting foam which contribute to poor crosslinking and splitting of the foam. Use of AFPs and AFGPs allow for the formation of a pore structure which has connected channels and thus allows for cohesion of the various sections of the foam. This feature improves the quality of the foams and enables the production of large foams. For example, when an AFP or combination of AFPs is freeze-dried with the biopolymer in high concentrations, e.g., about 0.2 to 0.5 mg/ml (about 124 μM), it dramatically reduces the normal foam pores until the foam resembles tightly packed long fibers. The foams produced using the AFPs in the freezing cycle can be employed, for example, as implants which direct specific cellular processes, e.g., through growth along the fibers.

After a single density foam is freeze-dried, it can be hydrated with, for example, a sterile aqueous buffer. If the hydrated single density foam is to be further shaped to have a selected form, e.g., it can be molded or formed in, on, or around a desired shape, e.g., it can be molded around a mandrel to form a tubular shape. Typically foams are then dried, e.g., air dried, at a temperature not greater than about 37° C.–40° C. under sterile conditions. At temperatures greater than about 37° C., the biopolymer in the foams will begin to denature, resulting in a double density foam that retains the fibers, walls, and two dimensional shape, but not the microcompartment sizes of the single density foams. The double density foam is stiff when dry and pliable when wet. It is resistant to tearing and to enzymatic digestion to a much greater extent that the single density foam. In contrast to the single density foam, the double density foam is a tight matrix which is preferred as a substrate for cells which normally grow on surfaces such as epithelial cells and endothelial cells. For example, the double density foam can be formed in the shape of a tube for use in reconstructing vessels or ducts or into a sheet and secured to large areas with sutures. Alternatively, the double density foam can be seeded with mesenchymal cells such as fibroblasts, muscle cells, chondrocytes, etc.

The resultant foam, whether single or double density, is removed, according to the invention, from support or shaping structures with which it is intimate contact and to which it typically adheres.

Fabrication of Collagen Matts

The present invention contemplates the maturation of biopolymer scaffolds in the form of biopolymer matt or biopolymer matt composites, e.g., resorbable biopolymer matt, for membranous or thick tissue applications, or as a filler material for tissue repair and tissue reconstruction which has a high strength to unit volume even before crosslinking. The invention also features biopolymer matt compositions comprising biopolymer matt and various layers of biopolymer foams, biocompatible constructs comprising biopolymer matt and extracellular matrix macromolecules, and methods for making and using the biopolymer matt, biopolymer matt composites, biopolymer matt compositions, and biocompatible matt constructs.

The biopolymer matt and biopolymer matt compositions can be used in vitro, for example, as model systems for research, or in vivo as prostheses or implants to replace damaged or diseased tissues or to provide scaffolds which, when occupied by cells, e.g., host cells, are remodeled to become functional tissues. In either case, the matt, matt composites, and matt compositions can be seeded with cells, e.g., mammalian cells, e.g., human cells, of the same type as those of the tissue which the matt, matt composites, or matt compositions is used to repair, reconstruct, or replace. Examples of tissues which can be repaired and/or reconstructed using the matt, matt composites, and matt compositions described herein include nervous tissue, skin, vascular tissue, cardiac tissue, pericardial tissue, muscle tissue, ocular tissue, periodontal tissue, connective tissue such as

bone, cartilage, tendon, and ligament, organ tissue such as kidney tissue, and liver tissue, glandular tissue such as pancreatic tissue, mammary tissue, and adrenal tissue, urological tissue such as bladder tissue and ureter tissue, and digestive tissue such as intestinal tissues. For example, the matt, matt composites, and matt compositions seeded with tissue specific cells are introduced into a recipient, e.g., a mammal, e.g., a human. Alternatively, the seeded cells which have had an opportunity to organize into a tissue in vitro and to secrete tissue specific biosynthetic products such as extracellular matrix proteins and/or growth factors which bond to the matt and matt compositions are removed prior to implantation of the matt or matt compositions into a recipient.

The present invention can utilize biopolymer matts, biopolymer matt composites, biopolymer matt compositions comprising biopolymer matt and biopolymer foam, biocompatible constructs comprising biopolymer matt and extracellular matrix macromolecules, and methods for making and using the matt, matt composites, and matt compositions. The biopolymer matt, matt composites, and matt compositions, in their native fibril structure, contain information to induce the repair or regeneration of damaged, diseased or missing tissue. Additional information for repair or regeneration can be added by mixing other informational macromolecules to the biopolymer matt, matt composites, and matt compositions. The biopolymer matt, matt composites, and matt compositions are fully resorbable when not reinforced by non-resorbable fibers and over time can be replaced by new normal pure host tissue. The biopolymer matt, matt composites, and matt compositions are more resistant to enzymatic breakdown than other collagen products of high information content, such as gels or foams. The biopolymer matt, matt composites, and matt compositions can be produced under physiological conditions, so living cells can be incorporated throughout the structure and on the completed form, thus yielding a living implant. The information content of the biopolymer matt, matt composites, and matt compositions can induce authentic healing and repair. For example, a living cell matt can replace the missing host tissue and its function immediately, and still be remodeled by authentic host tissue gradually with no interruption of tissue function. The biopolymer matt, matt composites, and matt compositions can be produced in a manner which gives them more strength than other collagen products of high information content. Therefore, they can be used in situations which requires an implant of strength.

A biopolymer is a naturally occurring polymeric substance formed from individual molecules in a biological system or organism. Biopolymers can also be man-made by manipulation of the individual molecules once obtained outside the biological system or organism. The biopolymer is suitable for introduction into a living organism, e.g., a mammal, e.g., a human. The biopolymer is non-toxic and bioabsorbable when introduced into a living organism and any degradation products of the biopolymer should also be non-toxic to the organism. The biopolymers of the invention can be formed into biocompatible forms, e.g., matt, matt composites, matt compositions, including biocompatible foams, biocompatible gels, biocompatible constructs which include biocompatible fibers, e.g., collagen fibers, biocompatible fabrics, e.g., collagen fabrics, all with or without other extracellular matrix macromolecules. Examples of molecules which can form biopolymers and which can be used in the present invention include collagen, laminin, elastin, fibronectin, fibrinogen, thrombospondin, gelatin, polysaccharides, poly-1-amino acids and combinations thereof. In one embodiment, a combination or mixture of one or more biopolymers can be used to form the biocompatible forms, e.g., fibers, matt, and matt compositions of the invention. For example, a combination of laminin and type IV collagen can be used to form the biopolymer fibers described herein. A preferred molecule for biopolymer production is collagen.

Preferred sources of molecules which form biopolymers include mammals such as pigs, e.g., near-term fetal pigs, sheep, fetal sheep, cows, and fetal cows. Other sources of the molecules which can form biopolymers include both land and marine vertebrates and invertebrates. In one embodiment, the collagen can be obtained from skins of near-term, domestic porcine fetuses which are harvested intact, enclosed in their amniotic membranes. Collagen or combinations of collagen types can be used in the matt and matt compositions described herein. Examples of collagen or combinations of collagen types include collagen type I, collagen type II, collagen type III, collagen type IV, collagen type V, collagen type VI, collagen type VII, collagen type VIII, collagen type IX, collagen type X, collagen type XI, collagen type XII, collagen type XIII, and collagen type XIV. A preferred combination of collagen types includes collagen type I, collagen type III, and collagen type IV. Preferred mammalian tissues from which to extract the molecules which can form biopolymer include entire mammalian fetuses, e.g., porcine fetuses, dermis, tendon, muscle and connective tissue. As a source of collagen, fetal tissues are advantageous because the collagen in the fetal tissues is not as heavily crosslinked as in adult tissues. Thus, when the collagen is extracted using acid extraction, a greater percentage of intact collagen molecules is obtained from fetal tissues in comparison to adult tissues. Fetal tissues also include various molecular factors which are present in normal tissue at different stages of animal development.

In a preferred embodiment, the biopolymer matt, matt composite, or matt composition is a collagen matt, collagen matt composite, or collagen matt composition. Collagen solutions can be produced by salt extraction, acid extraction, and/or pepsin extraction from the starting material. In a preferred embodiment, the collagen used is produced by sequentially purifying two forms of collagen from the same collagen-containing starting material. First, intact collagen is acid extracted from the starting material, the extract is collected and collagen is prepared as a collagen solution, e.g., by precipitating the collagen with sodium chloride and solubilizing the collagen in a medium having an acidic pH. Meanwhile, truncated collagen, i.e., collagen from which the telopeptides have been cleaved or partly cleaved leaving only the helical portion or the helical portion with some telopeptides, is extracted from the starting material using enzyme, e.g., an enzyme which is functional at an acidic pH, e.g., pepsin, extraction. Then, the collagen from this pepsin extract is purified separately by similar methods as from the first extract.

The biopolymer matt can also include a densely packed random array of biopolymer fibrils and has a high strength to unit volume and preserves the native structure of the biopolymer fibrils. Examples of molecules which can form biopolymer fibrils which can be used in the biopolymer matt include collagen, laminin, elastin, fibronectin, fibrinogen, thrombospondin, gelatin, polysaccharides, poly-1-amino acids and combinations of biopolymers. A preferred molecule for biopolymer production is collagen, e.g., porcine fetal collagen. In other embodiments, the biopolymer matt can include macromolecules necessary for cell growth,

morphogenesis, differentiation, or tissue building and combinations thereof, extracellular matrix particulates and/or cells.

The biopolymer matt, matt composite, or matt composition can be conditioned with cells prior to use in vitro or in vivo. Cell conditioning is an application-specific method used to speed integration of the matt, matt composite, or matt composition into its new function, to speed recovery of repair tissue and to direct authentic replacement of the damaged or missing tissue. Biopolymer matt, biopolymer matt composites, or biopolymer matt compositions can be used as a substrate for the growth of cells appropriate for the site of use. For example, for a biopolymer matt, biopolymer matt composite, or biopolymer matt composition used to repair bone defects as a periosteum, the conditioning cells would include, e.g., osteoblasts. For a biopolymer matt, biopolymer matt composite, or biopolymer matt composition used as pericardial membrane, the conditioning cells would include, e.g., mesothelial cells. For a biopolymer matt, biopolymer matt composite, or biopolymer matt composition used in the abdomen, the conditioning cells would include, e.g., mesothelial cells.

During conditioning, cells residing on biopolymer matt, biopolymer matt composite, or biopolymer matt composition deposit onto the matt, matt composite, or matt composition, macromolecules, such as protein products recognizable by the cells neighboring the defect at the site of matt, matt composite, or matt composition placement. The cell choice and thus the protein products can direct two things. They can direct the migration of the neighboring cells onto the matt, matt composite, or matt composition and the remodeling of the matt, matt composite, or matt composition material to replace the matt, matt composite, or matt composition with authentic covering tissue or the cell products will stimulate the regrowth of the tissue desired beneath the matt, matt composite, or matt composition while other cells remodel the matt, matt composite, or matt composition from the opposite side. After a period of time for the conditioning cells to deposit sufficient signaling and extracellular matrix molecules onto the matt, matt composite, or matt composition, the matt, matt composite, or matt compositions can be used as living implants to serve as living tissue equivalents or model tissue systems. Alternatively, cells of the matt, matt composite, or matt compositions can be killed by freezing or freeze drying the construct. Freeze drying eliminates living material, but leaves the deposited proteins in their natural states.

The biopolymer matt can be used alone, e.g., as a collagenous membrane for a periodontal barrier, or as a periosteal barrier to aid in bone repair. The biopolymer matt can also be used as a biopolymer composite by collecting sequential layers of different fibril slurry on the porous support and fusing these layers to each other. The biopolymer matt or biopolymer matt composites can also be used as a matt composition comprising a biopolymer matt and a biopolymer foam, e.g., as in the tissue repair of dura mater of the central nervous system. For example, a single density foam can be cast onto a finished matt to yield a structure with two layers of distinct characteristics, the matt layer of high density and low to no porosity and the foam layer with low density and high porosity. Single and double density biopolymer foams are described in U.S. Ser. No. 08/754,818, filed Nov. 21, 1996, the contents of which are incorporated herein by references in their entirety. Such implant sites consisting of compound tissue can be treated with matt compositions which include epi-meso- or endothelial cells on a matt surface and mesenchymal cells in the foam scaffold. For these applications, the low porosity matt side can minimize adhesions or fluid loss on one surface and the high porosity side can attract and support cell growth and differentiation required for healing. To further protect against adhesions or fluid loss in these applications and in applications requiring the use of matt alone, one can modify the surface of the matt. Modification can be accomplished biologically by growing and differentiating keratinocytes on one side of the matt to produce a stratum corneum. Matt compositions comprising one or more layers of biopolymer matt or biopolymer matt composites and more than one layer of single or double density biopolymer foams are also specifically contemplated herein.

As mentioned above, the matt can incorporate fiber structures, such as a single fibers, braids, or fabrics to achieve general reinforcement, directed reinforcement or to achieve directional cell growth. Examples of implants requiring such structures include skeletal replacements or temporary reinforcing structures. The matt can be cast in shapes other than sheets. It can be cast as tubes or orbs, such as spheres, to produce membranous structures which can contain material or liquids for specialized functions. Examples of implants made from matt, matt composite, or matt compositions include, for example, vessels, ducts, ureters, bladders and bone implants from matt cylinders filled with bone replacement material.

A matt composition comprising a matt and a single density foam, either with or without cell seeding, which is not freeze dried, can be used to build living tissue equivalents or model tissue systems. An example of this is the growth of dermal fibroblasts in the single density foam and the differentiated growth of keratinocytes on the porous surface matt layer for a skin model or a living implant system which quickly replaces lost function in critical situations and which can be cryopreserved for storage stockpiling. If not desired as a living implant system, the cell-laden developed complex can be freeze dried for later use as an implant which directs host tissue regrowth through information derived from the material the cultivated cells deposit onto the structures prior to freeze drying.

The biopolymers can be used to create matts, matt composites, or matt compositions which can be in any form or shape, e.g., strips, sheets, tubes, etc. In addition, the biopolymers can be used to create matts which can be supported by polymer mesh, e.g., a Teflon mesh, or used with tissue culture inserts for multiwell plates which can be used as molds in which matt, matt composites, and matt compositions of the invention can be formed on the polycarbonate membrane of the insert. Polymer meshes used with the matt, matt composites, and matt compositions of the invention can expose cells contained on and within the matt, matt composites, and matt compositions to the atmosphere as, for example, when the matt, matt composites, and matt compositions are used as skin equivalents to stimulate formation of a stratum corneum. Both the meshes and culture inserts have the advantage of providing a means for handling the matt, matt composites, and matt compositions without requiring actual contact with the matt, matt composites, or matt compositions. The forms and shapes in which the matt, matt composites, and matt compositions are made can mimic those of tissues or body parts to be replaced and thus can be used as prostheses or grafts which tissue cells remodel to promote regeneration of a replacement tissue in the recipient.

Macromolecules necessary for cell growth, morphogenesis, differentiation, and tissue building can also be added to the biopolymer molecules or to the biopolymer

fibrils to further promote cell ingrowtb and tissue development and organization within the matt. The phrase "macromolecules necessary for cell growth, morphogenesis, differentiation, and tissue building" refers to those molecules, e.g., macromolecules such as proteins, which participate in the development of tissue. Such molecules contain biological, physiological, and structural information for development or regeneration of the tissue structure and function. Examples of tbese macromolecules include, but are not limited to, growth factors, extracellular matrix proteins, proteoglycans, glycosaminoglycans and polysaccharides. Alternatively, the biopolymer matts, matt composites, and matt compositions of the invention can include extracellular matrix macromolecules in particulate form or extracellular matrix molecules deposited by cells or viable cells.

As used herein, tbe term "matt" refers to a biopolymer scaffold comprising a densely packed random array of biopolymer fibrils or bundles of fibrils or particles, e.g., collagen fibrils. Matts wbich bave been dried, as discussed previously, possess a wet tensile strength of at least 0.02 MPa with a preferred strength of greater than 1 MPa and have a collagenase resistance of at least 20 min per mg of collagen at a collagenase concentration of 10 units per 1 cm^2 of product. Typically the fibrils or bundles of fibrils are between about 0.01 μm and 50 μm in diameter and between about 0.0002 and 5.0 mm in length, preferably 0.1 μm to 20 μm wide and 0.01 mm to 3 mm long. Matts, whether dried or not, possess the following characteristics: (1) physically stable in aqueous solutions; (2) nontoxic to living organisms; (3) can serve as a substrate for cell attachment and growth; (4) approximately 0.01 mm to 20 mm thick, preferably 0.1 to 5.0 mm thick.

As used herein, the term "fibrils" refers to ordered multimers of molecules which create a fibrous overall structure. In the case of collagen fibrils, the collagen molecules are arranged in a quarter stagger, wbere each side-by-side association of molecules has an orderly shift of 25% (the head of one collagen molecule is arranged to be juxtaposed to the adjacent molecule 25% down the chain of that molecule). Fibrils, especially those of collagen often have a characteristic appearance by electron microscopy. Fibrils associate into bundles. Higher multiples of fibril bundles are fibers.

The term "matt composite" refers to a biopolymer form comprising sequential layers of biopolymer matt which are bonded to each other.

The term "matt composition" refers to a biopolymer composition comprising a matt, e.g., a biopolymer matt which is preferably resorbable, and optionally, one or more biopolymcr foams, e.g., a single or double density foam. Single and double density biopolymer foams are described in U.S. Ser. No. 08/754,818, filed Nov. 21, 1996, the contents of whicb are incorporated herein by references in their entirety.

The biopolymer foams can be single density or double density foams. As used berein, the term "foam" refers to a network of communicating microcompartments having biopolymer molecules and/or biopolymer filaments interspersed witbin the walls of the microcompartments. The language "single density foam" refers to a biopolymer foam baving at least two of tbe following characteristics: 1) it has microcompartments with the volume dimensions of x, y, and z wberein x=length, y=width, and z=beight and are substantially equal. Typically, x, y, and z range from about 1 μm to about 300 μm, preferably from about 20 μm to about 200 μm, more preferably from about 40 μm to about 150 μm, and most preferably from about 50 μm to about 100 μm; 2) it bas microcompartments with an average wall tbickness of less than about 10 μm; 3) it has microcompartmcnts with walls which include biopolymer fibers and/or filaments; 4) it is physically stable in aqueous solutions; 5) it is nontoxic to living organisms; and 6) it can serve as a substrate for cell attachment and growth. The single dcnsity foams retain tbcir structure when hydrated, for example, in aqueous buffer solution or tissue culture medium. In addition, the tbree dimensional structure of tbe single density foams can support the organization of cells seeded into them. Single density foams, when prepared from collagen and witbout cells, can be readily digested with collagenase, e.g., 0.1% collagenase. Examples of molccules which can form biopolymers which can be used in tbe single density biopolymer foams include collagen, alginic acid, polyvinyl alcohol, elastin, chondroitin sulfate, laminin, fibronectin, fibrinogen, and combinations of tbese biopolymers. A preferred biopolymer is collagen, e.g., porcine fetal collagen. In other embodiments, the single density biopolymer foams can include extracellular matrix particulates and/or cells.

As used herein, the language "double density foam" refers to a biopolymer foam having at least two of the following characteristics: 1) it has microcompartments with the volume dimensions of x, y, and z wherein x=length, y=width, and z=height, two of which are substantially equal and the third of which is decreased or diminished by a factor of at least about 10, and more preferably at least about 20 or more compared to the same dimension in the single density foam, and can range from about 1 μm to about 300 μm, preferably from about 20 μm to about 200 μm, more preferably from about 40 μm to about 150 μm, and most preferably from about 50 μm to about 100 μm; 2) it has microcompartments with an average wall thickness of less than about 10 μm; 3) it has microcompartments with walls which include biopolymer fibers and/or filaments; 4) it is physically stable in aqueous solutions; 5) it is nontoxic to living organisms; and 6) it can serve as a substrate for cell attacbment and growth. The double density foams, when prepared from collagen, are resistant to collagenase digestion to a greater degree than single density foams made from collagen, e.g., from about 3 to about 5 times or more, more resistant to 0.1% collagenase than single density foams. Double density foams prepared from collagen also have a higher collagen density per unit volume than the collagen content per unit volume of single density foams. When hydrated, the height of the double density foams is typically from about 0.2 mm to about 0.4 mm. Either surface of the double density foam provides a substrate suitable for plating epithelial, endothelial, and mesothelial cells which can form sheets. Mesenchymal cells can also be seeded onto the double density foams. The double density foams can be produced in the same sizes and same forms, e.g., in any form and in combination and bonded to a polymer mesh or as a multiwell plate insert, as the single density foams. Cells grown on both the single and double density foams of the invention have morphologies characteristic of cells of three dimensional tissues and can form normal intercellular relationsbips, i.e., intercellular relationships like those in the tissue from which they are derived or obtained. Preferred biopolymers for use in double density foams are described above as in single density foams. In other embodiments, tbe double density biopolymer foams can include extracellular matrix particulates and/or cells.

Either the surface of the matt, matt composites, or matt compositions can providc a substratc suitablc for plating epithelial, endothelial, and mesenchymal cells which can be

formed into sheets or other articles. Cells can also be seeded onto single or double density foams. Cells grown on biopolymer matts and biopolymer matt compositions have morphologies characteristic of cells of three dimensional tissues and can form normal intercellular relationships, i.e., intercellular relationships like those in the tissue from which they are derived or obtained.

Biopolymer nonwoven fabrics are typically composed of a collection of entangled biopolymer fibers of a selected size and density. Typically, the nonwoven biopolymer fabrics are produced by spooling dry biopolymer fiber onto a drum of circumference equal to that of the length of an individual fiber element. Spooling is continued until the number of wraps of fiber on the drum equals the number of pieces of fiber required for the fabric. A cut is then made across the wound fiber in a direction parallel to the drum axis and the fibers are removed from the drum. The fiber can then be crosslinked if it has not been previously crosslinked. The fiber is then dispersed in a volume of a phosphate buffer solution for a period of time to decrease its pH and soften the fiber. The fiber is transferred to a volume of water and agitated mechanically to produce entanglement of the fiber strands. The entangled fiber strands are sieved from the water onto a collection screen until they coat the screen in a mat of uniform density. The nonwoven fabric is then dried on the screen or after transfer to another surface, screen, or cell culture device. If desired, the nonwoven mat can be cut or punched into smaller shapes after drying.

Examples of materials suitable for matt reinforcing fibers, threads, braids, bundles of fibers, fabrics and/or nonwovens are materials which form biopolymers, resorbable polymers and non-resorbable polymers. Materials for biopolymers include collagen, alginic acid, laminin, elastin, gelatin, fibronectin, fibrinogen, thrombospondin, polysaccharides, poly-1 amino acids and combinations thereof. Materials for resorbable polymers include poly-α-hydroxyesters such as poly-1-lactic acid and poly-1-glycolic acid, polydioxinone, polyvinyl alcohol, surgical gut and combinations thereof. Examples of materials for non-resorbable polymers include silk, nylon, polytetrafluoroethylene, polypropylene, polyesters, polyurethanes and combinations thereof. Further combinations for fibers can be made by casting resorbable polymer fibers on non-resorbable polymers or casting biopolymer fibers on resorbable or nonresorbable fibers. A preferred material is collagen, preferably fetal porcine collagen.

Examples of implants requiring such reinforced structures include skeletal replacement, hernia repair or temporary reinforcing structures. For example, to repair a rotator cuff, strong fibers or sutures, preferably resorbable sutures, can be embedded within the matt structure at the time of depositing the fibril slurry to enable high tension to be placed on the finished product in the direction of reinforcement. These structures are described further herein.

Biocompatible constructs which include biopolymer matt or biopolymer matt compositions of the invention and extracellular matrix macromolecules are also specifically contemplated herein. Extracellular matrix macromolecules in soluble or particulate form dispersed or suspended in a biopolymer solution can also be applied onto and/or into the matt and matt compositions of the invention, thereby forming a matt, matt composite, or matt composition having extracellular matrix macromolecules or particulates. As used herein, the language "particulate form of extracellular matrix" refers to a fragment of an extracellular matrix derived from a tissue source formerly having living cells but which has been processed to remove the cells and to retain noncellular extracellular matrix factors such as, for example, growtb factors also proteins, proteoglycans, glycosaminoglycans necessary for cell growth, morphogenesis, and differentiation. Methods for forming extracellular matrix particulates for producing graft tissue are disclosed in U.S. patent application Ser. No. 07/926,885, filed Aug. 7, 1992, U.S. patent application Ser. No. 08/302,087, filed Sep. 6, 1994, and U.S. patent application Ser. No. 08/471,535, filed Jun. 6, 1995. The teachings of U.S. patent application Ser. Nos. 07/926,885, 08/302,087, and 08/471,535 are incorporated berein by reference.

The methods for forming extracellular matrix particulates include freezing a tissue source, e.g., a connective tissue source, baving living cells, whcreby the living cells are disrupted to form cell remnants consisting of, for example, cytoplasmic and nuclear components. The tissue source is then processed, e.g., by grinding, washing and sieving, to remove the cytoplasmic and nuclear components without removing extracellular matrix including macromolecules necessary for cell growtb, migration, differentiation, and morphogenesis. The extracellular matrix is freeze-dried and fragmented, e.g., cryomilled to produce particulates of defined sizes, to produce extracellular matrix particulates.

The extracellular matrix particulates can include extracellular matrix proteins. For example, extracellular matrix particulates obtained from skin include transforming growth factor β1, platelet-derived growth factor, basic fibroblast growth factor, epidermal growth factor, IGFl, bFGF, syndecan-1, decorin, fibronectin, collagens, laminin, tenascin, and dermatan sulfate. Extracellular matrix particulates from lung include PDGF, TGFβ1, bFGF, VEGF, syndecan-1, fibronectin, laminin, and tenascin. The extracellular matrix particulates can also include cytokines, e.g., growth factors necessary for tissue development. The term "cytokine" includes but is not limited to growth factors, interleukins, interferons and colony stimulating factors. These factors are present in normal tissue at different stages of tissue development, marked by cell division, morphogenesis and differentiation. Among these factors are stimulatory molecules that provide the signals needed for in vivo tissue repair. These cytokines can stimulate conversion of an implant into a functional substitute for the tissue being replaced. This conversion can occur by mobilizing tissue cells from similar contiguous tissues, e.g., from the circulation and from stem cell reservoirs. Cells can attach to the prostheses which are bioabsorbable and can remodel them into replacement tissues.

The matt, matt composite, and matt compositions can also be used as prostheses which can be introduced or grafted into recipients, e.g., such as mammalian recipients, e.g., humans. For example, the matt, matt composites, and matt compositions can be used as a prosthesis or to reconstitute, for example, the following types of tissue: nervous tissue, skin, vascular tissue, muscle tissue, connective tissue such as bone, cartilage, tendon, and ligament, kidney tissue, liver tissue, and pancreatic tissue. Tissue cells seeded into the matt, matt composites, and matt compositions can be obtained from a mammal, e.g., a human. If not added during matt formation, tissue cells are delivered to the matt, matt composites, and matt compositions by first suspending the cells in small volumes of tissue culture medium. The tissue culture medium which contains the cells can then be applied in drops to the matt, matt composites, or matt compositions. Alternatively, tbe matt, matt composites, or matt compositions can be placed in a vessel which contains the tissue culture medium and cells in suspension and which shakes such that the tissue culture medium containing the cells is

distributed throughout the matt, matt composites, or matt compositions. In another embodiment, tissue cells can be suspended in a biopolymer solution e.g., a collagen solution, at low concentrations, at a temperature of about 4° C. to 10° C., and at a pH of about 7.0. The solution containing the cells can then be delivered to the matt, matt composites, and matt compositions. As matt is warmed to 37° C., the biopolymer solution, e.g., collagen solution, forms a gel in the matt. As used herein, the term "gel" refers a network or mesh or biopolymer filaments together with an aqueous solution trapped within the biopolymer scaffold of biopolymer fibrils. An alginate gel for use as a delivery vehicle of cells to the matt, matt composites, or matt compositions of the invention can be produced by addition of calcium which causes polymerization at room temperature and at a neutral pH. Selected epithelial, endothelial, or mesothelial cells can then be plated onto the surface of the gel-filled matt, matt composite., or matt composition.

The biopolymer matt can be used alone, e.g., as a collagenous membrane for a periodontal barrier, or as a periosteal barrier to aid in bone repair. Alternatively, the biopolymer matt can be used in a biopolymer matt composition comprising a biopolymer matt and a biopolymer foam, e.g., as in the tissue repair of dura mater of the central nervous system, e.g., a single density foam can be cast onto the finished matt to yield a structure with two layers of distinct characteristics, the matt layer of high density and low to no porosity and the foam layer with low density and high porosity. Single and double density biopolymer foams are described in U.S. Ser. No. 08/754,818, filed Nov. 21, 1996, the contents of which are incorporated herein by references in their entirety. Implant sites requiring a compound tissue, e.g., skin made up of two tissues, the epidermis and the dermis, can be treated with matt compositions which include epi- meso- or endothelial cells on a matt surface and mesenchymal cells in the foam scaffold. For these applications, the low porosity matt side can minimize adhesions or fluid loss on one surface and the high porosity side can attract and support cell growth and differentiation required for healing. Modification can be accomplished, for example, biologically by growing and differentiating keratinocytes on one side of the matt to produce a stratum corneum. Matt compositions comprising one or more layers of biopolymer matt or biopolymer matt composites and more than one layer of single or double density biopolymer foams are also specifically contemplated herein.

As mentioned above, the matt can incorporate fiber structures, such as a single fibers, braids, bundles of fibers or fabrics to achieve general reinforcement, directed reinforcement or to achieve directional cell growth. Examples of implants requiring such structures include, e.g., skeletal replacements or temporary reinforcing structures.

The matt, matt composites, or matt compositions can be cast in shapes other than sheets. It can be cast as tubes or orbs, such as spheres, to produce membranous structures which can contain material or liquids for specialized functions. Examples of implants made from matt, matt composites, or matt compositions include, e.g., vessels, ducts, ureters, bladders and bone implants from matt cylinders filled with bone replacement material. As used herein, the term "bone replacement material" refers to material which can fill voids in bone and which can assist in bone repair. Examples of bone replacement material include, but are not limited to, autologous bone graft, bone powder, demineralized bone, calcium sulfates, and calcium phosphates, e.g., hydroxyapatites, brushites and octacalcium phosphate. A matt composition comprising a matt and a single density foam, either with or without cells, and which is not dried can be used to build living tissue equivalents or model tissue systems. An example of this is the growth of dermal fibroblasts in the single density foam and the differentiated growth of keratinocytes on the porous surface matt layer for a skin model or a living implant system which quickly replaces lost function in critical situations and which can be cryopreserved for storage stockpiling. If not desired as a living implant system, the cell-laden developed complex can be freeze dried for later use as an implant which directs host tissue regrowth through information derived from the material the cultivated cells deposit onto the structures prior to freeze drying as described for cell conditioning above.

The matt, matt composites, and matt compositions of the invention can also be formed into vascular prostheses in the form of a tube and can be seeded internally with smooth muscle cells delivered in a neutralized collagen solution that gels after delivery, externally with adventitial fibroblasts and on its luminal surface with endothelial cells. For example, a tubular matt can be formed by dispensing biopolymer fibril slurry into a tube whose inner diameter is the outer diameter of the desired matt. The tube is rolled continuously while the slurry dries. While drying occurs, fibrils deposit onto the tube. More slurry can be applied to the tube to add to matt thickness. When all fibrils have dried, the matt is removed as a seamless product from the tube. If reinforcement of the tubular matt is desired, fibers, bundles of fibers or fabrics can be positioned in the tube before or while the slurry is applied to the tube. These methods also apply to matts cast in other shapes, such as spheres, where the rolling would occur in more than one direction.

In a vascular prosthesis, the matt would become the inner layer inside of which the endothelial cells would be seeded. A single density foam would be cast around the matt for the albumenal substrate for the smooth muscle cells and adventitial fibroblasts.

Ligament implants, as multifilament forms of the biopolymers of the invention, can be enhanced with the matt, matt composites, and matt compositions of the invention to promote cell seeding. For example, continuous ligament multifilament structures can be produced with or without the addition of extracellular matrix particulates, to have selected characteristics. Ligament cells can then be delivered to the ligament which can be embedded in a matt casing. The ligament can then be mounted in a tubular tissue maturation chamber. After the ligament cells have attached to the ligament, the ligament is subjected to a regime of cyclical axial elongation resulting in stress, which is increased in magnitude as the ligament matures. The mature biopolymer ligaments can be used, for example, as ligament prostheses.

Dental implants can be formed from the matt, matt composites, and matt compositions of the invention. For example, the matt, matt composites, and matt compositions can be prepared as specialized dental implants for periodontal ligament repair and bone rebuilding. In one embodiment, the matt, matt composites, and matt compositions of the invention are prepared as apron shaped implants which can be fixed to a tooth by tying the strings of the apron around the tooth. In another embodiment, the matt, matt composites, and matt compositions are designed as covers of post extraction sockets filled with bone replacement material or collagen composition. In yet another embodiment, the matt, matt composites, and matt compositions are designed as bone replacement material-filled tubes to serve as alveolar ridge builders.

The apron shaped matt, which can be produced as a matt with low porosity or a matt composition including a double

density or quadruple density foam, i.e., a double density foam folded over on itself, for promoting periodontal ligament repair and bone rebuilding can be positioned between a gum flap and the alveolar bone in the area requiring periodontal ligament repair and bone rebuilding. The matt can be designed to block invasion by junctional epithelium of the cleaned and planed tooth zone. Periodontal ligament cells can then migrate into the matt, matt composite, or matt composition, bind to the matt, matt composite, or matt composition, and secrete extracellular matrix products into the matt, matt composite, or matt composition. The matt, matt composite, or matt composition can also be invaded by capillary endothelial cells and immune cells which provide defense against microbial assault. By excluding epithelium and by stimulating periodontal ligament cells, the matt, matt composite, or matt composition can promote regeneration of periodontal ligament and alveolar bone. The apron shaped dental implants can also be modified to include a bone replacement material as described herein. In one embodiment, the material can be included in an outpocketing of the apron which can be placed on the eroded alveolar bone. The bone replacement material provides pathways for invading bone cells. The apron shaped dental implant can also include extracellular matrix particulates generated from dental tissues. These extracellular matrix particulates provide the appropriate growth factors, e.g., bone and ligament specific growth factors, for promoting periodontal ligament cell and bone cell growth into the implant.

Alternatively, the matt, matt composites, and matt compositions of the invention can be prepared as post extraction socket covers. The matt can be used to cover the socket filler material which is inserted into sockets of extracted teeth. These socket fillers promote bone regeneration within the socket which, at a minimum, provides a foundation for a metal, e.g., titanium, fixture and subsequent application of a crown. The titanium or other material fixture can be anchored in a socket immediately after an extraction with calcium phosphate bone replacement material reinforced and covered or "tented" with one of the matt, matt composites, or matt compositions described herein as an apron. The socket fillers can also include extracellular matrix particulates generated from bone tissue or dental papilla. These extracellular matrix particulates provide the appropriate growth factors, e.g., bone specific growth factors, for promoting bone cell growth into the implant. In addition, in instances where the bony foundation for dental implants composed of metal does not provide adequate support for the metal implant, bone replacement material reinforced or strengthened with the foams and foam compositions of the invention can be used to reinforce the bony foundation.

In yet another embodiment, the matt, matt composites, and matt compositions can be designed as alveolar ridge substitutes or alveolar ridge builders. Alveolar ridge substitutes are used to provide underpinning for dentures. Typically, the alveolar ridge substitutes are designed as biopolymer matt tubes of the appropriate length which are filled with non-resorbable calcium phosphate bone replacement material to build up a mineralized platform along the alveolar ridge and to promote development of bone and a connective tissue framework around the calcium phosphate particles. The alveolar ridge builders of the invention have the same design as that of the alveolar ridge substitutes except that the matt tube is filled with resorbable forms of bone replacement material to promote bone development. The composition of the alveolar ridge builders promotes bone cell and blood capillary penetration leading to regrowth and restoration of the ridge prior to, for example, installation of a denture or a metal implant. The matt tube of the alveolar ridge builder can also include extracellular matrix particulates which promote alveolar ridge bone regeneration.

Similarly, the matt, matt composites, and matt compositions of the invention enriched, for example, with extracellular matrix particulates derived from organs e.g., cardiac tissue, bladder tissue, tissue from the small intestine, lung tissue, pancreatic tissue, hepatic tissue, skin tissue, and other organ tissue, can be seeded with analogous organ cells such as those of the endocrine pancreas, e.g., pancreatic islet cells, or those of the liver, e.g., hepatocytes, as means of promoting cell proliferation before and/or after implantation so that after implantation and vascularization of the cell-laden matt implant, a functional replacement organ develops.

Examples of cell types which have been successfully grown in and on the matt and matt compositions of the invention include mesenchymal cells, dermal fibroblasts, keratinocytes, osteoblasts, gingival fibroblasts, and tendon and ligament cells.

Biopolymer matts are described more fully in Attorney Docket No. TSS-028, filed Mar. 17, 1998, the contents of which are herein incorporated by reference.

Fabrication of Collagen Fibers

A biopolymer construct can be fabricated from biopolymer fibers. Methods and apparatus for fabricating biopolymer fibers are known to those of ordinary skill in the art as disclosed in U.S. Pat. No. 5,562,946, entitled "Apparatus and Method for Spinning and Processing Collagen Fiber," issued Oct. 8, 1996, and herein incorporated by reference.

It will thus be seen that the invention efficiently attains the objects set forth above, among those made apparent from the preceding description. Generally, disclosed herein are methods for exposing a biopolymer replacement tissue to a culturing, or maturation fluid, and for applying selected forces and/or stresses to the exposed tissue. Forces may be transmitted to the tissue by the maturation fluid. The invention functions in part by conditioning tissue in vitro in a manner designed to simulate selected in vivo conditions, i.e., the conditions under which a tissue grows, i.e., exposed to certain fluids, such as synovial fluid, and subjected to certain stresses, such as shear stresses.

Techniques disclosed herein include mounting a replacement tissue such that a surface of the tissue is spaced by a selected gap from a second surface, providing a maturation fluid in the gap for contacting at least a portion of each of the surfaces, and moving one of the surfaces relative to the other. Pressing the surfaces together subjects the tissue to a compressive pressure, which can be provided with or without relative motion between the surfaces. Tissue surfaces can slidingly and frictionally engage, or can frictionally and non-slidingly engage. Several embodiments of apparatus are disclosed for implementing the above techniques. However, these embodiments are intended as illustrative of apparatus for practicing the present invention and not as limiting. One of ordinary skill of the art, with knowledge of the present disclosure, can likely envision other embodiments, or variations of the disclosed embodiments, that encompass, and accomplish the purposes of, the present invention. For example, one of ordinary skill in the art could couple the transverse piston **154** in FIG. **2**A to an electromechanical actuator, rather than have the transverse piston **154** driven by fluid pressure. Accordingly, these variations and embodiments are considered within the spirit and scope of the invention.

It is also to be understood that the following claims are to cover all generic and specific features of the invention described herein, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween.

Having described the invention, what is claimed as new and desired to be secured by Letters Patent is:

1. An apparatus for maturing biopolymer tissue, comprising

first support means having a first surface for receiving and mounting a biopolymer tissue having a first tissue surface,

second support means having a second surface facing said first tissue surface,

fluid means for introducing a maturation fluid to one of said first tissue surface and said second surface, and

relative motion means coupled to at least one of said first and second support means for providing relative motion between the tissue received and mounted by said first surface and said second surface for subjecting the tissue to selected forces.

2. The apparatus of claim 1, wherein said fluid means includes means for maintaining the maturation fluid in communication with the biopolymer tissue received and mounted by said first surface.

3. The apparatus of claim 1, wherein said fluid means includes a fluid reservoir adapted for receiving and confining maturation fluid and for immersing at least a portion of the biopolymer tissue received and mounted by said first surface in the maturation fluid.

4. The apparatus of claim 1, wherein said fluid means includes nozzle means for directing a flow of maturation fluid towards the biopolymer tissue and said second surface.

5. The apparatus of claim 1, wherein said second support means is adapted for receiving and mounting a second biopolymer tissue.

6. The apparatus of claim 1, wherein said relative motion means includes means for translating the first tissue surface of biopolymer tissue received and mounted by said first surface of said first support means relative to said second surface of said second support means at speeds ranging between about 0.5 cm/sec and about 50 cm/sec.

7. The apparatus of claim 1, wherein said second surface of said second support means is spaced along a first axis from said first tissue surface of said first tissue received and mounted by said first support means, and wherein said relative motion means comprises means for translating, in a plane substantially transverse to said first axis, the first tissue surface relative to said second surface at speeds ranging between about 0.5 cm/sec and about 50 cm/sec.

8. The apparatus of claim 1, wherein at least one of said first and second support means includes a block forming one of said first and second surfaces, and wherein said relative motion means comprises a linear electromechanical actuator coupled to said block to cyclically translate said block in a plane substantially parallel to one of said first and second surfaces.

9. The apparatus of claim 1, wherein at least one of said first and second support includes means forming a bore in said housing, said bore mounting a piston for translation therein, and said relative motion means includes means forming fluid ports in communication with said bore and means for introducing a fluid to said bore via said fluid ports so as to translate said piston in said bore.

10. The apparatus of claim 1, wherein one of said first and second support means includes an inner rotatable cylinder and the other includes an outer hemi-cylinder, and said relative motion means includes means for rotating said rotatable cylinder.

11. The apparatus of claim 1, further including spacing means for spacing the first tissue surface of the biopolymer tissue received and mounted on said first surface from said second surface of said second support means by a selected gap.

12. The apparatus of claim 11, wherein said spacing means varies said gap between first tissue surface of the biopolymer tissue received and mounted on said first surface and said second surface by between about 0 mm and about 5 mm.

13. The apparatus of claim 11, wherein said spacing means includes an adjustment stage coupled to at least one of said first and second support means.

14. The apparatus of claim 11, wherein at least one of said first and second support means includes a piston disposed for translation in a bore, and said spacing means includes means forming fluid ports in said bore and means for varying the volume of a fluid in said bore, via said fluid ports, so as to translate said piston in said bore.

15. The apparatus of claim 1, further comprising compression means for subjecting at least the biopolymer tissue received and mounted by said first surface to a selected compressive pressure.

16. The apparatus of claim 15, wherein said compression means is adapted for generating a compressive pressure on the biopolymer tissue received and mounted on said first surface of said first support means of between about 0 psi and about 100 psi.

17. The apparatus of claim 15, wherein said compression means includes means for compressing the biopolymer tissue mounted on said first surface of said first support means and said second support means together.

18. The apparatus of claim 17, wherein said compression means further comprises platform means adapted for receiving a weight, said platform means coupled to at least one of said first and second support means for transmitting at least a portion of the gravitational force on the weight to said support means coupled thereto.

19. The apparatus of claim 17, wherein at least one of said first and second support means includes a piston disposed for travel in a bore having means forming at least one fluid port therein, and said compression means includes means for providing, via said fluid ports, a fluid to said bore such that said fluid has a selected pressure in said bore.

20. The apparatus of claim 5, further comprising compression means for compressively engaging the biopolymer tissue received and mounted by said first surface with the biopolymer tissue received and mounted by said second surface of said second support means such that a compressive pressure of between about 0 psi and about 100 psi acts on each of the biopolymer tissues.

21. The apparatus of claim 1, wherein the maturation fluid comprises a thixotropic fluid for transmitting shear forces between biopolymer tissue mounted and received on said first surface and said second surface.

22. The apparatus of claim 1, wherein the biopolymer tissue mounted on said first surface comprises a biopolymer foam.

23. The apparatus of claim 22, wherein said first support means is adapted for interposing bone cement between said first surface and the biopolymer tissue received and mounted thereon.

24. The apparatus of claim 23, wherein said bone cement comprises calcium phosphate.

25. The apparatus of claim 1, further including

housing means for confining a fluid, said housing means having

means forming a first bore having first and second ends and extending along a first longitudinal axis,

means forming a second bore having first and second ends and extending along a second longitudinal axis disposed at an angle relative to said first longitudinal axis, said second hore intersecting, at the second end thereof, said first bore hetween said first and second ends thereof,

wherein said first support means includes an extended piston disposed for translation in said first bore, said piston having an extended piston first face, an extended piston second face, and an interconnecting section therebetween, said interconnecting section having an outer surface, and

wherein said second support means including a transverse piston disposed in said second piston bore, said transverse piston having a transverse piston first face and a transverse piston second face.

26. The apparatus of claim 25, wherein said transverse piston second face is adapted for forming said second surface of said second support means, and said outer surface of said interconnecting section is adapted for forming at least a portion of said first surface of said first support means so as to face said transverse piston second face.

27. The apparatus of claim 26, further including compression means for applying a selected compressive force to at least the hiopolymer tissue mounted and received on said first surface.

28. The apparatus of claim 26, further including spacing means for translating said transverse piston in said second hore to vary the spacing between the first tissue surface of the hiopolymer tissue received and mounted on said first surface and said second surface.

29. The apparatus of claim 26, wherein said extended piston divides said first hore into a first volume bounded in part by said extended piston first face and said first end of said first bore, and a second volume bounded in part by said extended piston second piston face and said second end of said first bore.

30. The apparatus of claim 29, wherein said relative motion means includes means forming fluid ports in said first piston hore for transferring a fluid into one of said first and second volumes, and means for providing and for controlling the transfer of said fluid into said first and second volumes for selectively translating said extended piston in said first hore.

31. The apparatus of claim 29, wherein said first and second bores intersect so as to define a third volume bounded in part by said outer surface of said interconnecting section of said extended piston and said transverse piston second face, and said transverse piston divides said second bore into a fourth volume bounded in part by said first end of said second bore and said first face of said transverse piston, said apparatus further including means forming a fluid port in said second bore for transferring fluid into said fourth volume.

32. The apparatus of claim 31, further including means for controlling the transfer of a fluid to said fourth volume for adjusting said spacing between the biopolymer tissue received and mounted by said first surface and said second surface.

33. The apparatus of claim 31, further including compression means for engaging the biopolymer tissue received and mounted by said first surface and said second surface for applying a selected compressive pressure to at least the biopolymer tissue received and mounted by said first surface, said compression means including means for providing and transferring a fluid to said fourth volume so as to provide a selected fluid pressure in said fourth volume.

34. The apparatus of claim 31, wherein said means for introducing a maturation fluid includes means for transferring fluid between said third volume and at least one of said first, second and fourth volumes.

35. Apparatus for developing cartilage tissue, comprising

a fluid reservoir for holding a maturation fluid,

a first support elcment adapted for mounting a first biopolymer tissue having a first tissue surface,

a second support element adapted for mounting a second biopolymer tissue having a second tissue surface facing the first tissue surface of the first biopolymer tissue,

said first and second support elements heing adapted for immersing at least a portion of said first and second biopolymer tissue in the maturation fluid so as to contact the first and second hiopolymer tissue surfaces, and

translation means for translating said first support element relative to said second support element to translate said first tissue surface relative to said second tissue surface so as to develop the tissue.

36. The apparatus of claim 35, wherein said translation means comprises an electromechanical actuator coupled to said first support element.

37. The apparatus of claim 35, wherein said electromechanical actuator linearly reciprocates said first support element.

38. The apparatus of claim 35, further comprising compression means coupled to at least one of said first and second support elements for pressing together the first and second biopolymer tissue.

39. The apparatus of claim 35 wherein said first tissue surface is spaced from said second tissue surface so as to form a gap therebetween.

40. The apparatus of claim 39, further comprising variable spacing means for varying the spacing hetween the first and second biopolymer tissue.

41. Apparatus for promoting development of a biopolymer tissue, comprising

a reservoir for confining a maturation fluid,

a rotatable inner support cylinder having an outer circumferential surface adapted for mounting a first hiopolymer tissue having an outwardly facing tissue surface,

an arcuate support element spaced from said support cylinder and having an inner mounting surface adapted for mounting a second hiopolymer tissue having an inwardly facing tissue surface,

said rotatable support cylinder and said arcuate support element being spaced apart such that the outwardly facing tissue surface is spaced from and faces the inwardly facing tissue surface to form a gap therebetween, said rotatable support cylinder and said hemi-cylindrical support element being operatively arranged with said reservoir such that the maturation fluid held therein is introduced to said gap and contacts at least a portion of each of the inwardly and outwardly facing tissue surfaces, and

rotating means for rotating said rotatable inner support cylinder.

42. The apparatus of claim 41, wherein the outwardly facing tissue surface is spaced from the second inwardly facing tissue surface by between about 0 mm and about 5 mm.

43. The apparatus of claim 41, further comprising compression means for compressing together the inwardly and outwardly facing tissue surfaces.

44. The apparatus of claim 41, wherein said rotatable inner support cylinder and said arcuate support element are coaxially mounted.

45. The apparatus of claim 41, wherein said rotatable support cylinder and said arcuate support element are coaxially mounted and said gap is uniform.

46. The apparatus of claim 41 wherein said rotation means includes an electric motor having a shaft coupled to said rotatable cylinder.

47. Apparatus for the maturation of biopolymer tissue, comprising

a housing having

means forming a first bore having first and second ends and extending along a first longitudinal axis,

means forming a second bore having a first and second ends and extending along a second longitudinal axis disposed at an angle to said first longitudinal axis, said second bore intersecting, at the second end thereof, said first bore between said first and second ends thereof,

an extended piston disposed for translation in said first bore, said extended piston having an extended piston first face, an extended piston second face, and an interconnecting section therebetween, said interconnecting section having an outer surface adapted for receiving and mounting a first biopolymer tissue having a first tissue surface, and

a transverse piston disposed for translation in said second bore, said transverse piston having a transverse piston first face and a transverse piston second face, said second face being disposed for receiving and mounting a second biopolymer tissue having a second tissue surface facing the first tissue surface.

48. The apparatus of claim 47, wherein said extended piston divides said first bore into a first volume, bounded in part by said extended piston first face and said first end of said first bore, a second volume, bounded in part by said extended piston second piston face and said second end of said first bore.

49. The apparatus of claim 48, wherein said first and second bores intersect and define a third volume bounded in part by said outer surface of said interconnecting section of said extended piston and said transverse piston second face.

50. The apparatus of claim 49, wherein said transverse piston divides said second bore into a fourth volume bounded in part by said first end of said second bore and said first face of said transverse piston.

51. The apparatus of claim 50, further comprising means forming at least one fluid port in said first bore for transferring a first fluid to one of said first and second volumes.

52. The apparatus of claim 51, further comprising means forming at least one fluid port in said second bore for transferring a second fluid to said fourth volume.

53. The apparatus of claim 52, further comprising means for transferring fluid between said third volume and at least one of said first, second and fourth volumes.

54. The apparatus of claim 51, further comprising means for controlling the transfer of the first fluid to said first and second volumes for selectively translating said extended piston in said first bore.

55. The apparatus of claim 52, further comprising means for controlling the transfer of the second fluid to said fourth volume for selectively spacing the first biopolymer tissue from the second biopolymer tissue so as to form a selected gap between the first tissue surface and the second tissue surface.

56. The apparatus of claim 52, further comprising means for controlling the transfer of the second fluid to said fourth volume for selectively pressing the first biopolymer tissue against the second biopolymer tissue for providing a selected compressive pressure on at least one of the first and second biopolymer tissue.

57. A method of maturing biopolymer tissue, comprising the steps of

mounting a first biopolymer tissue having a first tissue surface to a first surface of a first support structure,

providing a second support structure having a second surface,

arranging said first and second support structures such that the second surface and the first tissue surface face each other,

introducing a maturation fluid to contact at least a portion of the second surface and at least a portion of the first tissue surface,

translating at least one of said first and second support structures relative to the other for applying selected forces, via the maturation fluid, to the first tissue.

58. The method of claim 57 further including the step of spacing the second surface of the second support structure from the first tissue surface of the first tissue mounted on the first support structure to form a gap between the said surfaces.

59. The method of claim 56, wherein the step of introducing a maturation fluid includes introducing the maturation fluid to the gap and includes the steps of providing a reservoir confining the maturation fluid and immersing at least a portion of the first biopolymer tissue and a portion of the second surface in the maturation fluid.

60. The method of claim 57, wherein the step of introducing a maturation fluid further comprises the step of introducing a thixotropic fluid.

61. The method of claim 57, wherein the step of introducing a maturation fluid further comprises the step of introducing hyaluronic acid.

62. The method of claim 57, wherein the step of introducing a maturation fluid further comprises the step of introducing synovial fluid.

63. The method of claim 58, wherein the step of spacing further comprises the step of separating the second surface and the first sheet of tissue such that at least a portion of the first tissue and at least a portion of the second surface are separated by a distance from between about 0 mm and about 5 mm.

64. The method of claim 57, wherein the step of translating further comprises the step of moving the first sheet of tissue relative to the second surface at speeds between about 0.5 cm/sec and about 50 cm/sec.

65. The method of claim 57, wherein the step of translating further comprises the step of linearly reciprocating the first biopolymer tissue relative to the second surface.

66. The method of claim 57, wherein the step of translating further comprises the step of rotating said first support structure relative to said second support structure.

67. The method of claim 57, further comprising the step of applying a selected compressive force to at least the first biopolymer tissue.

68. The method of claim 67, wherein the step of applying selected compressive forces further comprises the step of reducing the gap so as to press the second surface of said second support structure against the first biopolymer tissue.

69. The method of claim 57, further comprising the step of mounting a second biopolymer tissue on the second

support structure such that the second sheet of biopolymer tissue has a surface forming the second surface.

70. Apparatus for the maturation of biopolymer tissue, comprising

an inner cylinder extending longitudinally along a central axis and having an outer surface for receiving and mounting a first biopolymer tissue having an outwardly facing tissue surface,

an outer cylinder having a lumen therethrough and extendingly longitudinally along a second central axis substantially parallel to said first central axis, said outer cylinder having an outer wall having an inner face bounding said lumen, said inner face for receiving and mounting a second biopolymer tissue having an inwardly facing tissue surface,

said inner cylinder being disposed within said lumen such that said inwardly facing and outwardly facing tissue surfaces face each other,

means for providing a maturation fluid within said lumen for contacting said inwardly and outwardly facing tissue surfaces, and

at least a first rotational drive means for rotating at least one of said inner and outer cylinders for providing relative motion between said inwardly facing and outwardly facing tissue surfaces.

71. The apparatus of claim **70** wherein said inwardly facing tissue surface is spaced from said outwardly facing tissue surface by a gap.

72. The apparatus of claim **71** wherein the radius of curvature of the outer surface of said inner cylinder is less than the radius of curvature of said inner face of said outer wall of said outer cylinder and said inwardly facing tissue surface is spaced from said outwardly facing tissue surface by a non-uniform gap, said gap having a minimum magnitude and a maximum magnitude at points about the circumference of said tissue surfaces.

73. The apparatus of claim **71** including spacing means for varying the magnitude of said gap, said spacing means including means for varying the offset, in a direction transverse to said central axes, between said central axes of said cylinders.

74. The apparatus of claim **70** wherein said inwardly facing tissue surface engages said outwardly facing tissue surface at least along a line of engagement substantially parallel to said central axes.

75. The apparatus of claim **74** including a rotational support means for rotationally supporting the second of said inner and outer cylinders, such that rotationally driving one of said cylinders with said rotational drive means rotates the other of said cylinders due to frictional contact of said tissue surfaces at least along said line of engagement therebetween.

76. The apparatus of claim **74** including a second rotational drive means for rotationally driving the other of said cylinders such that said tissue surfaces frictionally and slidingly engage at least along said line of engagement.

77. The apparatus of claim **70** including means for compressing said outwardly facing tissue surface against said inwardly facing tissue surface at least along a line of engagement between said tissue surfaces.

78. The apparatus of claim **70** including translation means for translating said inner and outer cylinders relative to each other to vary the offset between said central axes in a plane transverse to said central axes such that a line of engagement of said inwardly and outwardly facing tissue surfaces is circumferentially varied about the inwardly facing tissue surface.

79. The apparatus of claim **78** wherein said inner cylinder is mounted to a shaft for rotation about said central axis of said inner cylinder,

said rotational drive means including said translations means, said translation means including means for translating said shaft, and

wherein frictional contact between said inwardly and outwardly facing tissue surfaces along said line of engagement rotates said inner cylinder about said first central axis.

80. An apparatus for maturing biopolymer tissue, comprising

a housing having an inner chamber,

an applicator having an arcuate pressure applying surface and adapted to be movable within the inner chamber,

first support means having a first surface for receiving and mounting a biopolymer tissue having a first tissue surface, said first surface having a concave surface feature formed therein, and

means for introducing a maturation fluid to the inner chamber,

whereby said applicator is movable relative to the tissue to apply a force thereto in the presence of the maturation fluid to mature the tissue.

81. The apparatus of claim **80** further comprising means for rotating said applicator.

82. The apparatus of claim **80** further comprising means for placing said applicator in contact with the tissue.

83. The apparatus of claim **80**, wherein said first support means comprises a piston cup assembly mounted within the housing chamber.

84. The apparatus of claim **83**, wherein said piston cup assembly further comprises one or more perfusion channels formed therein.

85. The apparatus of claim **84**, wherein said piston cup divides said inner chamber into an input chamber and an output chamber, said perfusion channels allowing fluid to pass between said input and output chambers, said apparatus further comprising sealing means for preventing fluid leakage about said piston cup and between said input and output chambers.

86. The apparatus of claim **84**, wherein said applicator has provided thereon a flange, said apparatus further comprising a stop ring mounted within said inner chamber of said housing, said stop ring having a flange adapted for engagement with the flange of said applicator to define a selected position of said applicator.

* * * * *

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(12) **United States Patent**
Cox

(10) **Patent No.: US 6,719,789 B2**
(45) **Date of Patent: Apr. 13, 2004**

(54) **REPLACEMENT HEART VALVE**

(75) Inventor: **James L. Cox**, Ladue, MO (US)

(73) Assignee: **3F Therapeutics, Inc.**, Lake Forest, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/153,286**

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(65) **Prior Publication Data**

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Related U.S. Application Data

(60) Continuation of application No. 09/924,970, filed on Aug. 7, 2001, which is a division of application No. 09/536,229, filed on Mar. 27, 2000, now Pat. No. 6,270,526, which is a division of application No. 09/288,998, filed on Feb. 3, 1999, now Pat. No. 6,092,529, which is a continuation of application No. 08/748,055, filed on Nov. 13, 1996, now abandoned, which is a division of application No. 08/459,979, filed on Jun. 2, 1995, now Pat. No. 5,713,950, which is a division of application No. 08/146,938, filed on Nov. 1, 1993, now Pat. No. 5,480,424.

(51) **Int. Cl.**[7] **A61F 2/06**
(52) **U.S. Cl.** **623/2.13**
(58) **Field of Search** 623/2.1, 2.12, 623/2.13, 2.16, 2.15

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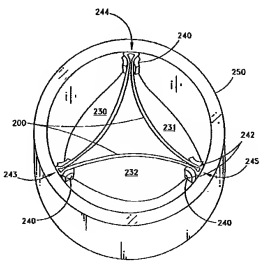
Primary Examiner—Corrine McDermott
Assistant Examiner—William H Matthews
(74) *Attorney, Agent, or Firm*—Jones Day

(57) **ABSTRACT**

A prosthetic heart valve has leaflets made of a thin and flexible material. The side edges of adjacent leaflets are sewn together so as to form a substantially tubular valve structure having an in-flow end and an out-flow end. Each of the leaflets is adapted to flex inwardly into and out of engagement with another leaflet so as to close and open the valve in response to force by blood pressure. The leaflets are configured so that a portion of the inner face of each leaflet is in a facing relationship with a portion of the inner face of an adjacent leaflet.

7 Claims, 5 Drawing Sheets



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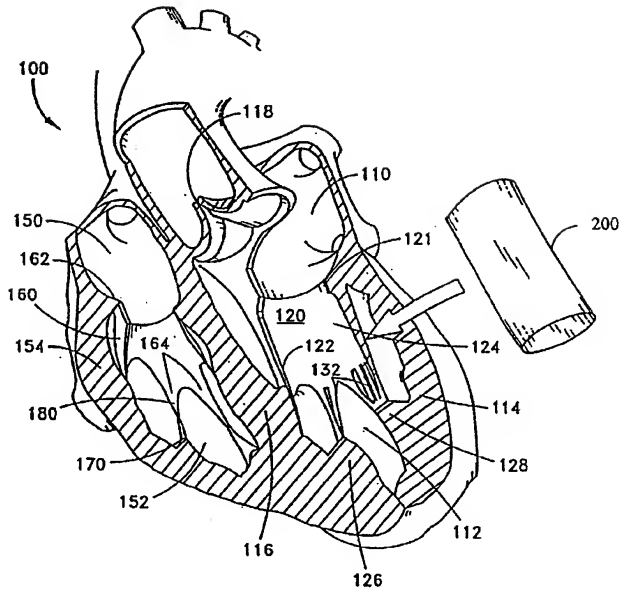
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FIG. 1

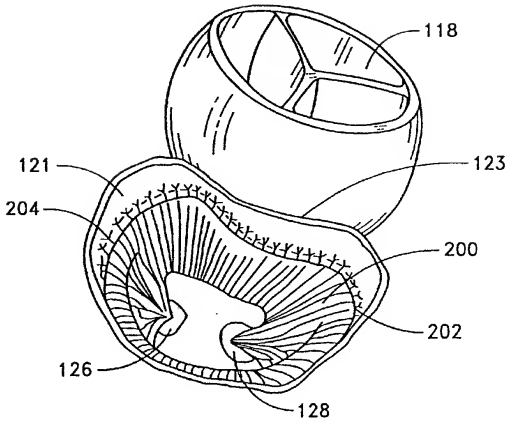

FIG. 2

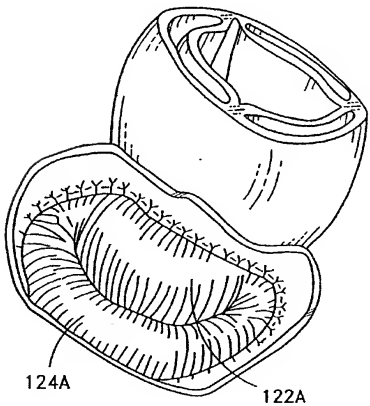

FIG. 3

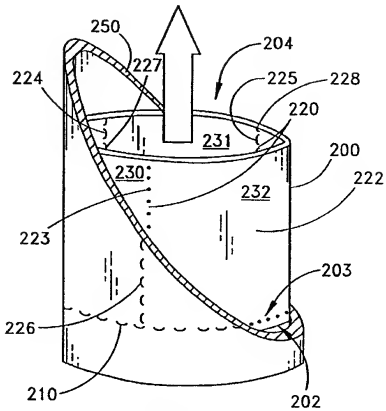
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FIG. 4

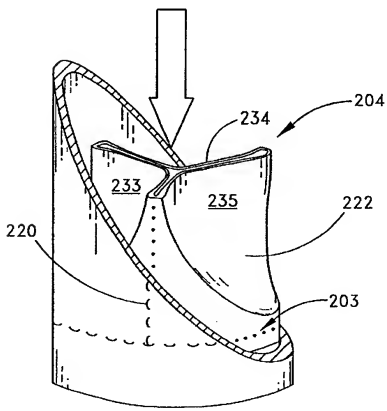
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FIG. 5

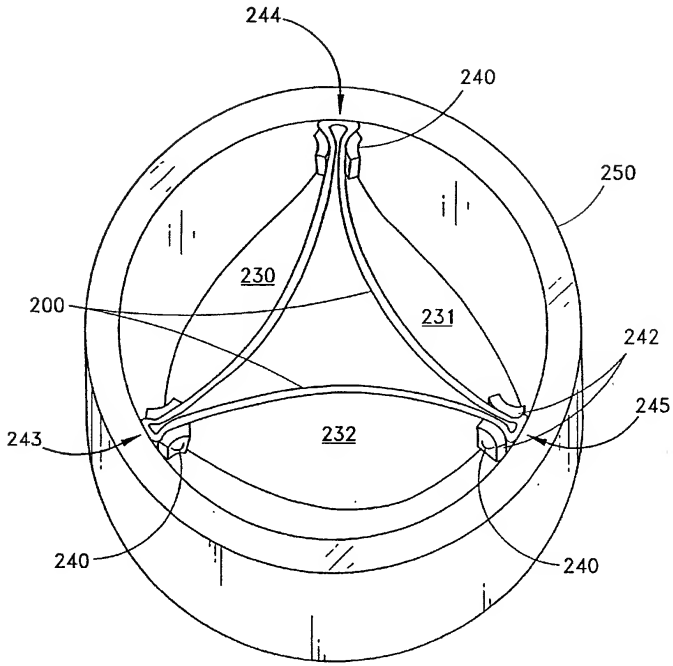

FIG. 6

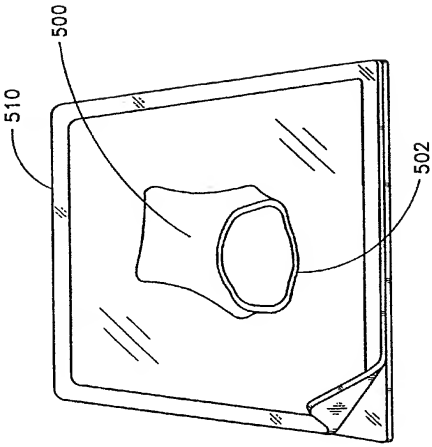

FIG. 8

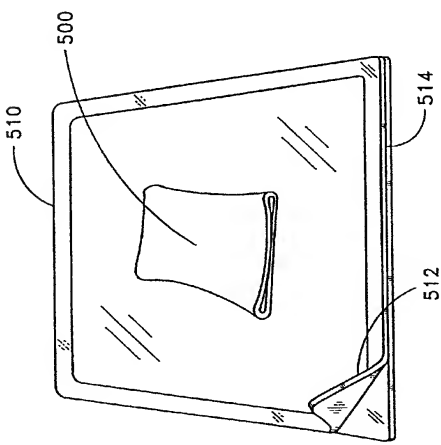

FIG. 7

REPLACEMENT HEART VALVE

RELATED APPLICATIONS

This is a continuation application based on prior U.S. Ser. No. 09/924,970, filed Aug. 7, 2001, which is a division of U.S. Ser. No. 09/536,229, filed on Mar. 27, 2000, now U.S. Pat. No. 6,270,526, which is a division of U.S. Ser. No. 09/288,998, filed on Feb. 3, 1999, now U.S. Pat. No. 6,092,529, which is a continuation of U.S. Ser. No. 08/748,055, filed on Nov. 13, 1996 NOW ABN, which is a division of U.S. Ser. No. 08/459,979, filed on Jun. 2, 1995, now U.S. Pat. No. 5,713,950, which is a division of U.S. Ser. No. 08/146,938, filed Nov. 1, 1993, now U.S. Pat. No. 5,480,424. The entire disclosure of each of the aforementioned patents and applications are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention is in the field of replacement heart valves.

Anatomy of Normal Heart Valves

There are four valves in the heart that serve to direct the flow of blood through the two sides of the heart in a forward direction. On the left (systemic) side of the heart are: 1) the mitral valve, located between the left atrium and the left ventricle, and 2) the aortic valve, located between the left ventricle and the aorta. These two valves direct oxygenated blood coming from the lungs, through the left side of the heart, into the aorta for distribution to the body. On the right (pulmonary) side of the heart are: 1) the tricuspid valve, located between the right atrium and the right ventricle, and 2) the pulmonary valve, located between the right ventricle and the pulmonary artery. These two valves direct de-oxygenated blood coming from the body, through the right side of the heart, into the pulmonary artery for distribution to the lungs, where it again becomes re-oxygenated to begin the circuit anew.

All four of these heart valves are passive structures that they do not themselves expend any energy and do not perform any active contractile function. They consist of moveable "leaflets" that are designed simply to open and close in response to differential pressures on either side of the valve. The mitral and tricuspid valves are referred to as "atrioventricular valves" because of their being situated between an atrium and ventricle on each side of the heart. The mitral valve has two leaflets and the tricuspid valve has three. The aortic and pulmonary valves are referred to as "semilunar valves" because of the unique appearance of their leaflets, which are more aptly termed "cusps" and are shaped somewhat like a half-moon. The aortic and pulmonary valves each have three cusps.

Since the physiological struetures of native mitral and tricuspid valves and native aortic and pulmonary valves are important to this invention, they are depicted in FIG. **1**, whieh contains a cross-seetional cutaway depiction of a normal human heart **100** (shown next to heart **100** is a segment of tubular tissue **200** whieh will be used to replace the mitral valve, as described below). The left side of heart **100** eontains left atrium **110**, left ventricular chamber **112** positioned between left ventricular wall **114** and septum **116**, aortie valve **118**, and mitral valve assembly **120**. The components of the mitral valve assembly **120** include the mitral valve annulus **121**, whieh will remain as a roughly circular open ring after the leaflets of a diseased or damaged valve have been removed; anterior leaflet **122** (sometimes called the aortic leaflet, since it is adjacent to the aortic region); posterior leaflet **124**; two papillary muscles **126** and **128** which are attached at their bases to the interior surface of the left ventricular wall **114**; and multiple chordae tendineae **13** which couple the mitral valve leaflets **122** and **124** to the papillary muscles **126** and **128**. There is no one-to-one chordal connection between the leaflets and the papillary muscles; instead, numerous chordae are present, and chordae from each papillary musele **126** and **128** attach to both of the valve leaflets **122** and **124**.

The other side of the heart contains the right atrium **150**, a right ventricular chamber **152** bounded by right ventricular wall **154** and septum **116**, and a tricuspid valve assembly **160**. The tricuspid valve assembly **160** comprises a valve annulus **162**, three leaflets **164**, papillary museles **170** attached to the interior surface of the right ventrieular wall **154**, and multiple chordae tendineae **180** which eouple the tricuspid valve leaflets **164** to the papillary muscles **170–174**.

As mentioned above, the mitral valve leaflets **122** and **124**, and tricuspid valve leaflets **164** are all passive structures; they do not themselves expend any energy and do not perform any active contractile funetion. They are designed to simply open and close in response to differential pressures on either side of the leaflet tissue. When the left ventricular wall **114** relaxes so that the ventricular ehamber **112** enlarges and draws in blood, the mitral valve **120** opens (i.e., the leaflets **122** and **124** separate). Oxygenated blood flows in a downward direction through the valve **120**, to fill the expanding ventricular cavity. Once the left ventricular cavity has filled, the left ventricle contracts, eausing a rapid rise in the left ventricular cavitary pressure. This causes the mitral valve **120** to close (i.e., the leaflets **122** and **124** re-approximate) while the aortic valve **118** opens, allowing the oxygenated blood to be ejected from the left ventricle into the aorta. The chordae tendineae **132** of the mitral valve prevent the mitral leaflets **122** and **124** from prolapsing back into the left atrium **110** when the left ventricular chamber **114** contracts.

The three leaflets, chordae tendineae, and papillary muscles of the tricuspid valve function in a similar manner, in response to the filling of the right ventricle and its subsequent contraction.

The cusps of the aortic valve also respond passivcly to pressure differentials between the left ventricle and the aorta. When the left ventricle contracts, the aortic valve cusps open to allow the flow of oxygenated blood from the left ventricle into the aorta. When the left ventricle relaxes, the aortic valve cusps reapproximate to prevent the blood which has entered the aorta from leaking (regurgitating) back into the left ventricle. The pulmonary valve cusps respond passively in the same manner in response to relaxation and contraction of the right ventricle in moving de-oxygenated blood into the pulmonary artery and thenee to the lungs for re-oxygenation. Neither of these semilunar valves has associated chordae tendineae or papillary muscles.

In summary, with relaxation and expansion of the ventricles (diastole), the mitral and tricuspid valves open, while the aortic and pulmonary valves close. When the ventricles contract (systole), the mitral and tricuspid valve close and the aortic and pulmonary valves open. In this manner, blood is propelled through both sides of the heart.

The anatomy of the heart and the strueture and terminology of heart valves are described and illustrated in detail in numerous reference works on anatomy and eardiae surgery, including standard texts such as *Surgery of the Chest* (Sabiston and Spencer, eds., Saunders Publ., Philadelphia) and ***Cardiac Surgery*** by Kirklin and Barrett-Boyes.

Pathology and Abnormalities of Heart Valves

Heart valves may exhibit abnormal anatomy and function as a result of congenital or acquired valve disease. Congenital valve abnormalities may be so severe that emergency surgery is required within the first few hours of life, or they may be well-tolerated for many years only to develop a life-threatening problem in an elderly patient. Acquired valve disease may result from causes such as rheumatic fever, degenerative disorders of the valve tissue, bacterial or fungal infections, and trauma.

Since heart valves are passive structures that simply open and close in response to differential pressures on either side of the particular valve, the problems that can develop with valves can be classified into two categories: 1) stenosis, in which a valve does not open properly, or 2) insufficiency (also called regurgitation), in which a valve does not close properly. Stenosis and insufficiency may occur concomitantly in the same valve or in different valves. Both of these abnormalities increase the workload placed on the heart, and the severity of this increased stress on the heart and the patient, and the heart's ability to adapt to it, determine whether the abnormal valve will have to be surgically replaced (or, in some cases, repaired) or not.

In addition to stenosis and insufficiency of heart valves, surgery may also be required for certain types of bacterial or fungal infections in which the valve may continue to function normally, but nevertheless harbors an overgrowth of bacteria (a so-called "vegetation") on the leaflets of the valve that may flake off ("embolize") and lodge downstream in a vital artery. If such vegetations are on the valves of the left side (i.e., the systemic circulation side) of the heart, embolization results in sudden loss of the blood supply to the affected body organ and immediate malfunction of that organ. The organ most commonly affected by such embolization is the brain, in which case the patient suffers a stroke. Thus, surgical replacement of either the mitral or aortic valve (left-sided heart valves) may be necessary for this problem even though neither stenosis nor insufficiency of either valve is present. Likewise, bacterial or fungal vegetations on the tricuspid valve may embolize to the lungs (resulting in a lung abscess) and therefore, may require replacement of the tricuspid valve even though no tricuspid valve stenosis or insufficiency is present. With the exception of congenital pulmonary valve stenosis or insufficiency, it is unusual for a patient to develop an abnormality of the pulmonary valve that is significant enough to require surgical repair or replacement.

Currently, surgical repair of mitral and tricuspid valves is preferred over total valve replacement when possible, although often the valves are too diseased to repair and must be replaced. Most abnormalities of the aortic valve require replacement, although some efforts are now being made to repair insufficient aortic valves in selected patients. Valve repair and valve replacement surgery is described and illustrated in numerous books and articles, including the texts cited herein.

Current Options for Heart Valve Replacement

If a heart valve must be replaced, there are currently several options available, and the choice of a particular type of prosthesis (i.e., artificial valve) depends on factors such as the location of the valve, the age and other specifics of the patient, and the surgeon's experiences and preferences. Available prostheses include three categories of valves or materials: mechanical valves, tissue valves, and aortic homograft valves. These are briefly discussed below; they are illustrated and described in detail in texts such as Replacement Cardiac Valves, edited by E. Bodnar and R. Frater (Pergamon Press, New York, 1991).

Artificial Mechanical Valves

Mechanical valves include caged-ball valves (such as Starr-Edwards valves), bi-leaflet valves (such as St. Jude valves), and tilting disk valves (such as Medtronic-Hall or omniscience valves). Caged ball valves usually are made with a ball made of a silicone rubber (Silastic™) inside a titanium cage, while bi-leaflet and tilting disk valves are made of various combinations of pyrolytic carbon and titanium. All of these valves are attached to a cloth (usually Dacron™) sewing ring so that the valve prosthesis can be sutured to the patient's native tissue to hold the artificial valve in place postoperatively. All of these mechanical valves can be used to replace any of the heart's four valves. No other mechanical valves are currently approved for use by the FDA in the U.S.A.

The main advantage of mechanical valves is their long-term durability. Their main disadvantage is that they require the patient to take systemic anticoagulation drugs for the rest of his or her life, because of the propensity of mechanical valves to cause blood clots to form on them. If such blood clots form on the valve, they may preclude the valve from opening or closing correctly or, more importantly, the blood clots may disengage from the valve and embolize to the brain, causing a stroke. The anticoagulant drugs that are necessary to prevent this are expensive and potentially dangerous in that they may cause abnormal bleeding which, in itself, can cause a stroke if the bleeding occurs within the brain.

In addition to the mechanical valves available for implantation today, a number of other valve designs are described and illustrated in a chapter called "Extinct Cardiac Valve Prostheses," at pages 307–332 of Replacement Cardiac Valves (Bodnar and Frater, cited above). Two of the "extinct" valves which deserve attention as prior art in the subject invention are the McGoon valve (pp. 319–320) and the Roe-Moore valve (pp. 320–321). Both of these involve flexible leaflets made of an elastomer or cloth coated with polytetrafluoroethylene (PTFE, widely sold under the trademark TEFLON), mounted inside a cylindrical stent. Although both were tested in humans, they were never commercialized and apparently are not being actively studied or developed today.

Artificial Tissue Valves

Most tissue valves are constructed by sewing the leaflets of pig aortic valves to a stent (to hold the leaflets in proper position), or by constructing valve leaflets from the pericardial sac (which surrounds the heart) of cows or pigs and sewing them to a stent. The stents may be rigid or slightly flexible and are covered with cloth (usually a synthetic material sold under the trademark Dacron™) and attached to a sewing ring for fixation to the patient's native tissue. The porcine or bovine tissue is chemically treated to alleviate any antigenicity (i.e., to reduce the risk that the patient's body will reject the foreign tissue). These tri-leaflet valves may be used to replace any of the heart's four valves. The only tissue valves currently approved by the FDA for implantation in the U.S.A. are the Carpentier-Edwards Porcine Valve, the Hancock Porcine Valve, and the Carpentier-Edwards Pericardial Valve.

The main advantage of tissue valves is that they do not cause blood clots to form as readily as do the mechanical valves, and therefore, they do not absolutely require systemic anticoagulation. Nevertheless, many surgeons do anticoagulate patients who have any type of artificial mitral valve, including tissue valves. The major disadvantage of tissue valves is that they lack the long-term durability of mechanical valves. Tissue valves have a significant failure

rate, usually appearing at approximately 8 years following implantation, although preliminary results with the new commercial pericardial valves suggest that they may last longer. One cause of these failures is believed to be the chemical treatment of the animal tissue that prevents it from being antigenic to the patient. In addition, the presence of the stent and sewing ring prevents the artificial tissue valve from being anatomically accurate in comparison to a normal heart valve, even in the aortic valve position.

Homograft Valves

Homograft valves are harvested from human cadavers. They are most commonly aortic valves but also occasionally include pulmonic valves. These valves are specially prepared and frozen in liquid nitrogen, where they are stored for later use in adults for aortic valve replacement, or in children for pulmonary valve replacement. A variant occasionally employed for aortic valve replacement is to use the patient's own pulmonary valve (an autograft) to replace a diseased aortic valve, combined with insertion of an aortic (or pulmonary) homograft from a cadaver to replace the excised pulmonary valve (this is commonly called a "Ross procedure").

The advantage of aortic homograft valves is that they, appear to be as durable as mechanical valves and yet they do not promote blood clot formation and therefore, do not require anticoagulation. The main disadvantage of these valves is that they are not available in sufficient numbers to satisfy the needs of patients who need new aortic or pulmonary valves. They also cannot be used to replace either the mitral valve or tricuspid valve. In addition, they are extremely expensive and much more difficult to implant than either mechanical or tissue valves. The difficulty in implantation means that the operative risk with a homograft valve is greater in a given patient than it is with either a mechanical or tissue valve. An additional problem is that in June 1992, the FDA reclassified homograft valves as an experimental device, so they are no longer available on a routine basis.

Principles of Artificial Heart Valve Construction

All artificial heart valves are designed to optimize three major physiologic characteristics and one practical consideration. The three major physiologic characteristics are (1) hemodynamic performance, (2) thrombogenicity, and (3) durability. The practical consideration involves ease of surgical implantation.

Multiple factors impact on each of these potential problems in the development of artificial valves. As a result, the advantage of artificial valve A over artificial valve B in one area is typically counterbalanced by valve B's advantage in another area. If one artificial heart valve were clearly superior in all aspects to all other artificial valves in all four of these areas, it would be the only artificial valve used.

Artificial Mechanical Valves

The hemodynamic performance of mechanical heart valves has been satisfactory but not optimal, especially in the smaller sizes. All previously constructed mechanical heart valves have had some type of obstructing structure within the flow orifice of the valve when the valve is in the open position. For example, bi-leaflet valves, such as the St. Jude valve, have two bars across the orifice and in addition, the leaflets themselves are within the orifice when the valve is in the open position. Single-leaflet disc valves, such as the Medtronic-Hall valve, have a central bar and strut mechanism that keep the leaflet in place. The Bjork-Shiley valves have either one or two struts that span the valve orifice in addition to the partially-opened disc itself. The omniscience valve has the partially opened disk itself in the valve orifice when open, and the Starr-Edwards caged-ball valve has both the ball and the cage within the flow orifice of the valve in the open position. All of these structures decrease the hemodynamic performance of the mechanical valves.

Such obstructions also interfere with the normal flow patterns within and around the mechanical valve and therefore, promote thrombosis. More importantly, all artificial surfaces are thrombogenic (clot-promoting) to a greater or lesser degree. The only completely non-thrombogenic (non-clot-promoting) surface that exists is the layer of viable endothelial cells that line the interior of all the body's vascular surfaces, including the inside of the heart chambers and the native valve leaflets. Therefore, any metal or plastic material, no matter how highly polished, will have some level of thrombogenicity unless the surface of the artificial material can be covered with endothelial cells. It is for this reason that all patients with artificial mechanical heart valves must be permanently anticoagulated.

The major advantage of mechanical valves over tissue valves is long-term durability. Mechanical valve construction has been based on sophisticated engineering principles that have proven to be sound in terms of providing devices that are extremely resistant to wear and structural failure. Nevertheless, structural failure of mechanical valves does occur and it is the major reason for the recent withdrawal from the market of two commercially available mechanical valves (the Bjork-Shiley Concavo-Convex™ single disc valve and the Duramedics™ bi-leaflet valve).

Artificial Tissue Valves

Under the best of circumstances (i.e., replacement of the aortic valve), the construction of artificial tissue valves has been based on the concept that if the artificial valve can be made to approximate the anatomy (form) of the native valve, then the physiology (function) of the artificial valve will also approximate that of the native valve. This is the concept that "Function Follows Form." For example, the manufacturers of all artificial porcine valves first re-create the form of a native human aortic valve by: 1) harvesting a porcine aortic valve, 2) fixing it in glutaraldehyde to eliminate antigenicity, and 3) suturing the porcine valve to a stent to hold the three leaflets in place. In other words, the primary goal in the construction of these artificial valves is to reproduce the form of the human aortic valve as closely as possible. The assumption is made that if the artificial valve can be made to look like the human aortic valve, it will function like the human aortic valve (i.e., proper function will follow proper form). The same assumption is also followed for commercially available pericardial valves.

In the case of mitral or tricuspid valve replacement, even the dubious concept of "function follows form" has been discarded since the same artificial valves that are designed to look like the aortic valve are used to replace the mitral and tricuspid-valves. In other words, no attempt at all is made to reproduce even the form of these native valves, much less so their function.

Thus, in the case of artificial valves to be used for aortic valve replacement, the dubious concept of "function follows form" has dictated the construction of all artificial tissue valves during the 30 years of their development and use. Even worse, no discernable underlying concept at all has been used in terms of the artificial valves used to replace the mitral and tricuspid valves.

The "Function Follows Form" concept has several limitations and appears to be a fundamental shortcoming which underlies the present construction of all artificial tissue valves. In the first place, it simply is not possible to recreate the exact anatomy (form) of a native heart valve utilizing present techniques. Although homograft (human cadaver)

and porcine aortic valves have the gross appearance of native aortic valves, the fixation process (freezing with liquid nitrogen, and chemical treatment, respectively) alters the histologic (microscopic) characteristics of the valve tissue. Porcine and bovine pericardial valves not only require chemical preparation (usually involving fixation with glutaraldehyde), but the leaflets must be sutured to cloth-covered stents in order to hold the leaflets in position for proper opening and closing of the valve. A recent advance has been made in this regard by using "stentless" porcine valves that are sutured directly to the patient's native tissues for aortic valve replacement, but the problem of chemical fixation remains. In addition, these stentless artificial valves cannot be used for mitral or tricuspid valve replacement.

Perhaps the major limitation of the "Function Follows Form" concept is that no efforts have been made previously to approximate the form of either the mitral valve or the tricuspid valve. If animal tissue valves are used to replace either of these native valves, the tri-leaflet porcine aortic valve prosthesis or the tri-leaflet bovine pericardial valve prosthesis is normally used. In doing so, even the faulty concept of "Function Follows Form" is ignored, since there are no artificial valves available for human use that approximate the anatomy (form) of the native mitral or tricuspid valves.

The nearest attempt at reproducing the function of the native mitral valve was reported by Mickleborough et al in 1989. These tests involved the use of commercially-prepared sheets of pericardial tissue from cows, which had been treated with glutaraldehyde before storage and shipping. A longitudinal suture line was used to convert the flat sheet of tissue into a cylinder, then two triangular regions were removed from one end of the cylinder, to generate two flaps. The inlet end was sutured to the mitral valve annulus, while the two tissue flaps at the carved outlet end were sutured to the papillary muscles.

The mitral valve disclosed by Mickleborough et al suffers from a drawback which is believed to be important and perhaps even crucial to proper valve functioning. In a properly functioning natural valve, the anterior leaflet does not have its center portion directly attached to the anterior papillary muscle via chordae. Instead, the anterior leaflet is attached to both the anterior and posterior papillary muscles, via chordae that are predominantly attached to the peripheral edges of the leaflet. In the same manner, a native posterior leaflet is attached to both the anterior and posterior papillary muscles, via chordae that are predominantly attached to the peripheral edges of the leaflet. As a result, the line of commissure (closure) between the two mitral leaflets when the valve is closed during systole is oriented in roughly the same direction as an imaginary line that crosses the tips of both papillary muscles. This orientation of the leaflets and papillary muscles is shown in illustrations such as page 11 of Netter 1969. This natural orientation can be achieved in the valve of the subject invention as depicted in FIGS. **2** and **3**, discussed below.

By contrast, the replacement valve described by Mickleborough et al alters and distorts the proper orientation of the replacement leaflets. Mickleborough's approach requires each sculpted leaflet to be trimmed in away that forms an extended flap, which becomes a relatively narrow strand of tissue near its tip. The tip of each pericardial tissue strand is sutured directly to a papillary muscle, causing the strand to mimic a chordae tendineae. Each strand extends from the center of a leaflet in the Mickleborough et al valve, and each strand is sutured directly to either an anterior and posterior papillary muscle. This requires each leaflet to be positioned directly over a papillary muscle. This effectively rotates the leaflets of the Mickleborough valve about 90° compared to the leaflets of a native valve. The line of commissure between the leaflets, when they are pressed together during systole, will bisect (at a perpendicular angle) an imaginary line that crosses the peaks of the two papillary muscles, instead of lying roughly along that line as occurs in a native valve.

There has been no indication since the publication of Mickleborough et al 1989 that their approach is still being studied (either by them, or by any other research team), and there has been no other indication during the intervening years that their approach is likely to lead to a valve replacement technique for actual use in humans.

It should be noted that one of the primary goals of Mickleborough and her associates apparently was to propose a new way to maintain continuity between the valve annulus and the papillary muscles. It was first proposed about 30 years ago (by C. W. Lillehei and perhaps by others as well) that proper muscle tone of the left ventricular wall, and proper postoperative ventricular functioning, required a tension-bearing connection between the mitral valve annulus and the papillary muscles on the inside of the ventricular wall. This suggestion was widely ignored in the design of replacement mitral valves, which required excision of the chordae tendineae without making any effort to provide a substitute that would keep the ventricular wall coupled to the valve annulus. However, various studies (such as Rittenhouse et al 1978, David 1986, Hansen et al 1987, and Miki et al 1988) continued to indicate that the tension-conveying role of the chordae was important to proper ventricular function. Based on those studies, Mickleborough et al apparently were attempting to create and propose a new valve design that could accomplish that goal. They did indeed accomplish that goal, and the apparent lack of any follow-up or commercialization of their design presumably was due to other problems, such as the altered orientation of the leaflets in their design.

A different approach to creating artificial tissue valves is described in articles such as Love and Love 1991, and in U.S. Pat. No. 5,163,955 (Calvin et al 1992) and U.S. Pat. No. 4,470,157 (Love 1984). In that research, surgeons harvested a piece of pericardial tissue from the same animal that was to receive the artificial valve. Such tissue, if harvested from the same human body that will receive the implant, is referred to as autologous or autogenous (the terms are used interchangeably, by different researchers). Using a cutting die, the pericardial tissue was cut into a carefully defined geometric shape, treated with glutaraldehyde, then clamped in a sandwich-fashion between two stent components. This created a tri-leaflet valve that again resembles an aortic or pulmonary valve, having semilunar-type cusps rather than atrioventricular-type leaflets. These valves were then tested in the mitral (or occasionally tricuspid) valve position, using sheep.

Although those valves were structurally very different from the valves of the subject invention, the Love and Love article is worth attention because it discusses chemical fixation. They used glutaraldehyde treatment even though their tissue source was from the same animal and was therefore non-antigenic, because earlier reports and tests had suggested that some types of untreated autologous tissue suffer from thickening and/or shrinkage over time. Love and Love suggested that glutaraldehyde can help such tissue resist such changes, apparently by forming crosslinking bonds that tend to hold adjacent collagen fibers in a fixed-

but-flexible conformation. This use of glutaraldehyde fixation as a treatment to reduce shrinkage or other physical distortion (as distinct from using it as a method of reducing tissue antigenicity) is an old and well-established technique for treating non-autologous tissue, but whether it is also beneficial for treating autologous tissue has not yet been extensively evaluated. The effects of chemical fixation of intestinal or other tubular tissue used to create heart valves as described herein can be evaluated by routine experimentation.

Another report describing the use of autologous tissue to reconstruct mitral valves is Bailey et al 1970. However, Bailey et al focused on repairing rather than replacing mitral valves, usually by cutting an incision into one or both leaflets and then inserting a segment of tissue into the incision to enlarge the leaflet(s).

Physiologic Factors and in Utero Development

The subject invention relates to a method of using tubular starting material to replace any of the four heart valves during cardiac surgery. This approach is supported by and consistent with a fundamental principle of native heart valve function, which either went unrecognized in previous efforts to develop replacement valves, or which was sacrificed and lost when compromises were required to adapt available materials to surgical requirements.

The basic principle, which deserves repeated emphasis because it has been so widely disregarded by other efforts in this field, is that Form Follows Function. In one manifestation of this principle, if an artificial valve can be created that can truly function like a native valve, its resultant form will be similar to that of the native valve.

A highly important observation by the Applicant that contributed to the recognition of the pervasive and overriding importance of this principle was the following: the entire cardiovascular system, including the heart, begins in utero as a single, relatively straight tube of tissue. Anatomical drawings depicting the in utero development of the heart are available in numerous scientific publications and books, including Netter 1969. As shown in those figures (or similar figures available in other medical reference works), the so-called "heart tube" is readily discernible by the 23rd day of gestation. This tube will eventually develop into the entire cardiovascular system of the body. The tissue that exists between the portion of the tube destined to become the ventricles, and the portion that will become the atria, is where the mitral and tricuspid valves will ultimately form. This region of tissue is in a tubular form.

The heart tube undergoes a process of convolution beginning at approximately 25 days gestation. This convolution of the heart tube forms what is called the "heart loop" and is responsible for the aortic valve ultimately coming to lie adjacent to the mitral valve. When a mature mitral valve is viewed from the atrial side, the anterior portion of the mitral valve annulus is relatively flat. This distortion of the original roundness of the mitral annulus is caused by the presence of the aorta against the anterior mitral valve. It is also the reason that the anterior leaflet of the mitral valve is contiguous with the aortic valve annulus. Finally, it explains why accessory atrioventricular connections (accessory pathways) that occur in the Wolff-Parkinson-White syndrome never occur in this portion of the mitral valve annulus; this is the only portion of the entire atrioventricular groove on either side of the heart where the atrium and ventricle were never contiguous during fetal development.

By approximately 56 days gestation, the heart tube development reaches a stage that displays a first constricted tube region between the primordial right atrium and the primordial right ventricle (this portion of the tube will become the tricuspid valve) and a second constricted tube region between the primordial left atrium and primordial left ventricle (the future mitral valve).

As the developing heart of a fetus undergoes various convolutions, septations, and compartmentalizations, the tissues that are to eventually become the heart valves maintain their tubular structure. Prior to the onset of fetal heart function, portions of the walls of these tubular structures undergo a process of dissolution, leaving behind only those portions of the original tubes that are necessary for the proper functioning of the heart. This dissolution also affects the ventricular walls as they rapidly enlarge in size; if it did not, the walls would become prohibitively thick as the physical size of the heart increased, and the heart could not function effectively as a pump since it would become simply a large mass of ventricular muscle.

The dissolution process also operates on the tubular constrictions that will become the four heart valves. In the case of the semilunar valves (the aortic and pulmonary valves), the necessary functional remnants are the three cusps, which are the remains of the functioning portion of a simple tube. This principle is strengthened by the fact that although frequent reference is made to the pulmonic or aortic valve "annulus", knowledgeable anatomists are quick to point out that there is no such anatomical structure. The thickened tissue that is commonly referred to as the "annulus" of these valves is simply the flexion point of the three cusps, the remnants of a simple tube that is fixed at three points distally and subjected to uniform pressure on its outside, resulting in collapse of the tube on the three sides between the points of distal fixation, which in turn, results in three nearly identical cusps. All tissue other than these moveable and functional cusps has undergone the normal process of dissolution as the aorta and pulmonary artery have enlarged, leaving behind only that tissue recognized as the cusps of these semilunar valves.

At the mitral and tricuspid valves locations, the dissolution process leaves behind the valve leaflets, chordae tendineae, and papillary muscles in both the right ventricle (tricuspid valve) and left ventricle (mitral valve). In other words, that portion of the original tube that is necessary for the development of the native heart valves is spared the dissolution process and the rest of the tube dissolves away. The valve leaflets are tube remnants, which are attached circumferentially to the fibrous annulus of the heart at their base and attached by chordae tendineae (additional tube remnants) at their free edges to papillary muscles (still more tube remnants) inside the ventricles. The leaflets, chordae tendineae, and papillary muscles of each the two A-V valves represent the necessary functional remnants of the original in utero tubular structures of the heart.

Using "Form Follows Function" as a basic guiding principle, the present invention is based on the realization that a tubular structure having proper size and suitable material characteristics, if placed inside a mitral or tricuspid valve annulus after excision of the native valve (or inside an aorta or pulmonary artery, as described below) will function exactly like the normal valve in that position, assuming proper fixation of the inlet and outlet ends of the tube. The "Form Follows Function" principle predicts that if the intended function of a replacement valve is to emulate the performance and function of a native mitral or tricuspid valve, then the form of a replacement valve—the structure and appearance of the replacement valve—should resemble the form of a native mitral or tricuspid valve. Since the native valves are generated from tubular starting material

during fetal development, this principle further suggests that replacement valves should also be generated from tubular material.

This principle is given added support by the results that were observed in an artificial tissue valve that had been implanted into the mitral valve position in a human heart. The Applicant learned of these results during a presentation by Professor Donald Ross of the National Heart Hospital and Brompton Hospital (London, England), the cardiac surgeon who had performed that surgery. The implanted valve was originally a commercially available trileaflet tissue valve that was implanted into the mitral position in a 35-year-old female. The trileaflet valve had been constructed using fascia late tissue (a relatively tough and flexible layer of tissue that normally surrounds certain types of muscles) which had been sewn into a circular stent. After 5 years, the artificial valve had to be removed because its leaflets had become calcified and immobile, resulting in both mitral stenosis and mitral insufficiency. Upon exposing the artificial valve during the removal surgery, the surgeon was struck by the similarity in shape and appearance of the diseased trileaflet valve to a normal mitral valve. The commissures of the three leaflet artificial tissue valve had fused in a manner so that two leaflets had been formed: one large anterior leaflet, and one smaller posterior leaflet, as seen in a native mitral valve. Furthermore, the commissure between the two leaflets when the patient's valve was closed by back pressure closely resembled the semi-circular commissure formed by leaflets in a native mitral valve.

During the presentation by Professor Ross, the Applicant witnessed a picture showing how the three-leaflet artificial valve had been converted into a bi-leaflet valve during the course of five years inside a human heart. It became clear to the Applicant that the patient's heart had been attempting to make the valve conform to the heart's functional needs.

Prior to witnessing that presentation, the Applicant had already been considering the question of whether tubular tissue might be useful for creating replacement heart valves. After seeing Prof. Ross's photographs, which provided strong physiological confirmation of the "Form Follows Function" principle, the Applicant began to carry out experiments to assess the possibility of using tubular tissue to replace heart valves. In a simple mechanical test, he obtained some highly flexible rubber tubes by cutting off the fingers of surgical gloves, then he sculpted the finger tubes to resemble the leaflets of mitral or tricuspid valves, then he sutured the sculpted rubber tubes inside of slightly larger tubes made of Dacron™. An internal rubber tube was secured proximally around the entire periphery of a tube, to emulate a valve annulus, and the sculpted rubber flaps at the distal ends were coupled to the tube walls by means of loose suture strands that emulated chordae tendineae. When cyclical pressure was generated by attempting to blow and then suck air through the tube, the interior rubber leaflets opened and closed in a manner that looked identical to natural mitral or tricuspid leaflets opening and closing. This provided additional confirmation of the "Form Follows Function" principle.

The physiologic principle that the functional components of native heart valves are the remnants of simple tissue tubes, and the idea of using tubular structures to replace defective heart valves, has been completely ignored in the design and construction of all replacement valves in use today. Indeed, although "Form Follows Function" is a well-respected principle in fields such as engineering or evolutionary studies, it is often disregarded among medical researchers, some of whom apparently seem to feel that efforts to sever or reverse this relationship represent triumphs of technology over nature. As an example, kidney dialysis machines, which look nothing like normal kidneys, are a purely technological, non-natural solution; they use a completely artificial form to generate and provide a certain needed function. However, as any dialysis patient would attest, they fall far short of being truly optimal.

In a similar manner, all artificial heart valves in use today, whether tissue or mechanical, have been designed based on the belief that either: 1) function can be forced to follow form (aortic and pulmonary valve replacement), or 2) neither function nor form of the native valve can be reproduced, so a replacement valve (either tissue or mechanical) must merely function as a one-way passive valve (mitral and tricuspid valve replacement). In the case of artificial tissue valves, the form of an artificial valve is established first, in the hope that the valve will function in a manner similar to a native valve. In the case of artificial mechanical valves, the disruption of the interaction between form and function goes even farther, and the caged balls, hinged flappers, and other devices in mechanical valves have even less physical similarity to native valves. However, the problems in both of these approaches are evident in the limitations suffered by every type of replacement valve that is in use today.

There is another way to express the concept of "Form Follows Function" which may help explain it to people who would point to mechanical heart valves, dialysis machines, and other non-natural forms that have been used to mimic the function of body parts. In such examples, function is forced to follow form. In crude and simple terms, the function of a heart valve is merely to allow flow in one direction only. Any type of mechanical check valve with a caged-ball or flapper-and-seat design can provide that level of function.

However, when the long-term aspects of heart valve function are also taken into account (including the functions of providing low hemolysis, low turbulence, avoiding calcification, etc.), it becomes clear that artificial forms cannot fully provide those functions. The best and perhaps only way to provide a replacement valve with the complete, long-term functionality of a natural heart valve is by giving proper deference to the relationship between function and form.

This principle can be stated as, "Form and function form a cycle." Each follows the other, but each also precedes and affects the other. If either half of this cycle is violated or disrupted, it will create problems that will stand in the way of an optimally functional, reliable, durable system with minimal hemolysis, turbulence, and calcification. On a short-term basis, function can be forced to adapt to an unnatural form; however, any such short-term solution will be plagued by problems and limitations over the long run. The problems and shortcomings of current mechanical replacement valves are a clear and direct demonstration of this principle.

The following series can help to illustrate the principle, "Form and function form a cycle." First, a form is created: tubular tissue is used to create a new mitral valve. This form then creates a function: the new valve allows flow in only one direction, from the atrium to the ventricle. This function, in turn, creates another form: the leaflets of the new mitral valve will close in a "smile" configuration resembling a native mitral valve during closure. This secondary form then creates a secondary function: the new valve will provide good long-term use and low levels of turbulence, hemolysis, calcification, and leaflet stress. Form and function form a cycle, and this cycle cannot be disrupted by injecting and

imposing an artificial, unnatural form in the heart without impeding the ability of proper form and proper function to interact with, support, and enhance each other.

In addition, certain items of evidence suggest that conventional replacement tissue valves, which cause high levels of turbulence, contribute to the important problem of leaflet calcification. The correlation between high turbulence and leaflet calcification is discussed below.

OBJECTS OF THE INVENTION

On the basis of the physiological facts, observations, and principles described above, and on the basis of experiments carried out by the Applicant, it appears that if heart valves are damaged or diseased to the point of requiring replacement, they should be replaced by tubular structures which function like native heart valves.

Accordingly, one object of this invention is to provide a replacement heart valve including a tubular segment of material that more closely resembles the construction and function of a native heart valve.

This and other objects and advantages of the invention will become clear as the invention and certain preferred embodiments are described below and in the drawings.

SUMMARY OF THE INVENTION

In accordance with one aspect of the present invention, a prosthetic heart valve comprises at least two cusps comprised of a thin and flexible material. Each cusp has an inner surface and an outer surface and is attached to another cusp along a longitudinal suture line. Each of the cusps is adapted to flex inwardly into and out of engagement with another cusp so as to close and open the valve in response to force by blood pressure. A portion of the inner surface of one cusp is in a facing relationship with a portion of the inner surface of another cusp adjacent the longitudinal suture line when the valve is open.

In accordance with another aspect, the present invention discloses a method for making a prosthetic heart valve. A section of substantially flat, flexible material is provided, and at least two leaflets are cut out of the flat material. Each of the leaflets has an inner face, an outer face, an in-flow end, an out-flow end, and side edges. The side edges of adjacent leaflets are sewn together so as to form a substantially tubular valve structure having an in-flow end and an out-flow end. Each leaflet is configured so that a portion of the inner face of each leaflet is in a facing relationship with a portion of the inner face of an adjacent leaflet.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a cutaway depiction showing the mitral valve on the left (systemic) side of the heart and the tricuspid valve on the right (pulmonary) side of the heart, showing a tubular segment adjacent to the heart, having a size and configuration that will render the segment suitable for implantation as a replacement mitral valve.

FIG. 2 depicts a top view (from the left atrium) of a tubular tissue valve sutured into the mitral position, showing the orientation of the anterior and posterior leaflets in relation to the anterior and posterior papillary muscles.

FIG. 3 shows a top view of the tubular mitral valve during systolic contraction of the ventricle. The two leaflets are pressed against each other in a natural "smile" configuration; this closure (approximation) of the leaflets prevents blood from flowing back into the atrium.

FIG. 4 depicts a tubular segment that has been inserted into an aorta or pulmonary artery, to create a semilunar valve with cusps.

FIG. 5 depicts a semilunar valve as described herein, in a closed position.

FIG. 6 depicts a configuration that can be used if desired to secure tubular tissue inside an aorta in a configuration in which the cusps of the valve are pinched together adjacent to the arterial wall.

FIG. 7 depicts a tubular segment of intestinal or synthetic material, enclosed within a sealed pouch that maintains sterility of the tubular segment.

FIG. 8 depicts a tubular tissue segment of intestinal or synthetic material which has been attached to an annuloplasty ring, enclosed within a sealed sterile pouch.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

This invention comprises a method of using tubular material, such as a tubular segment of synthetic material, or a segment of small intestinal submucosal (SIS) tissue, to replace heart valves (i.e., aortic or pulmonary valves) during cardiac surgery.

As used herein, "tubular starting material" refers to material that is harvested from a human or animal body in tubular form (such as intestinal tissue), and to synthetic material that is synthesized, molded, woven, or otherwise created in tubular form. Tubular starting material is distinct from flat material that has been secured by means such as longitudinal suturing, into tubular form.

This approach to using tubular material is substantially different from all artificial valves (mechanical or tissue) that are available for human use today. It is based upon the recognition of a fundamental principle of native heart. valve structure and function, which either has gone unrecognized or which has been sacrificed and lost when compromises were required to adapt available materials to surgical requirements. The basic principle, as described in the Background section, is that "Form Follows Function." If an artificial valve can be created that truly functions like a native valve, its resultant form will, of necessity, be similar to that of a native valve.

To assess and display the "Form Follows Function" principle mathematically, a flexible tubular segment was created in a three-dimensional CAD-CAM program, which was run on a computer in the Applicants research laboratory. The tube segment was affixed, at certain designated points, to the interior wall of a cylindrical flow conduit. One end (corresponding to the inlet) of the flow conduit and flexible tube were flattened on one side, and the flexible tube inlet was fixed around the entire inner circumference of the flow conduit. The other end (the "outlet") of the flexible tube was fixed at only two opposed points inside the flow conduit. An external force of 120 mm Hg (corresponding to the pressure generated in the left ventricle during systolic contraction of the ventricle) was applied to the outlet end of the flexible tube, and all unattached areas of the flexible tube were allowed to flex and move according to the mathematical deformations and constraints that occurred as a result of the imposed conditions. The program used an iterative finite-element algorithm to determine where each square in an imaginary grid on the surface of the flexible tube would be located. It was allowed to run to completion, which took approximately 12 hours. At the end of these calculations, the wall of the tube was visually depicted by the computer, and the resultant shape of the tube perfectly resembled the shape of a mitral valve when closed by back-pressure in a left ventricle.

A similar CAD-CAM analysis was performed for an aortic (or pulmonary) valve in which the inlet end of the

flexible tube was fixed circumferentially around the inlet of the flow conduit, and the other end of the flexible tube was fixed at 3 equidistant points around the circumference of the conduit. The external pressure applied to the outside of the tube was 80 mm Hg, corresponding to the arterial pressure exerted on normal aortic valve leaflets during diastole. Again, the resultant shape of the tube after 12 hours of mathematical deformation appeared to exactly mimic a natural aortic valve.

Until the CAD-CAM studies had been performed, the relationship of the principle of "Form follows Function" to the form and function of native human heart valves was only a hypothesis. However, the fact that the simple tubes, faxed in a known anatomic manner, were deformed by physiologic pressures into a shape that exactly mimicked the shape of native heart valves confirmed two aspects of the hypothesis in a convincing manner: 1) native heart valves do in fact function like the sides of compressed tubes when they close, and 2) the engineering principle of Form Following Function is applicable to native human heart valves.

To the best of the Applicant's knowledge, the significance of the in utero development of native heart valves as the remnants of simple tissue tubes, and the principle of using tubular structures to replace defective heart valves in an effort to reproduce the function of the native valves, has not previously been recognized or disclosed. The most closely related effort at creating artificial heart valves were described in Mickleborough et al 1989, which was discussed in the Background section. However, they did not use tubular material as the starting material; instead, they used bovine pericardial material, which is effectively flat. That approach required the used of animal tissue that had been treated with chemicals (glutaraldehyde) to reduce its antigenicity.

The approach described in Mickleborough et al 1989 also required the creation of a suture line to convert the flat pericardial tissue into a quasi-tubular structure. This created certain problems and risks, since a longitudinal suture line requires additional handling of the pericardial material by surgeons. This additional handling would need to be done after the patient's chest and heart have been surgically opened, therefore increasing the time during which the patient needs to be kept on cardiopulmonary bypass (CPB). As is well known; any increase in the length of time of artificial circulatory support is adverse, and any reduction of the time required for keeping a patient on CPB is beneficial. In addition, the creation of a longitudinal suture line during a mitral or tricuspid valve replacement might increase the risk of tearing the leaflet material at the suture points, and the risk of thrombosis. For both of these reasons, the use of tubular starting material (such as intestinal tissue) as described herein, rather than flat starting material, is advantageous, provided that the intestinal tissue segment has a diameter compatible with the valve being created. As discussed below, if the diameter of a patient's autologous intestinal segment is not compatible with the diameter of the annulus of a heart valve being replaced (which is likely when aortic or pulmonary valves are being replaced), a pre-treated packaged segment of SIS tissue having the desired diameter from an animal (such as a pig) or a human cadaver can be used to avoid the need for using a longitudinal suture line to convert flat material into tubular material.

In comparing the subject invention to the prior art of Mickleborough et al, it should also be kept in mind that the approach used by Mickleborough et al caused the anterior and posterior leaflets of their replacement valve to be rotated roughly 90° compared to the native leaflets in a native mitral valve. By contrast, the subject invention allows the creation of mitral leaflets having a natural orientation. This factor was discussed in the Background section and is depicted in FIGS. **2** and **3**.

Use of Intestinal Tissue

The harvesting, preparation, and use of intestinal tissue, for creating replacement heart valves, is described in detail in the two above-cited parent applications (Ser. No. 08/146,938, now U.S. Pat. No. 5,480,424, and Ser. No. 08/459,979, now U.S. Pat. No. 5,713,950), which are incorporated by reference in their entirety. The following discussion regarding implantation of intestinal tissue which has been properly harvested and prepared also applies directly to implantation of synthetic and any other material.

Methods of Implantation

The SIS tissue segment can be implanted in a mitral valve position using any of several methods. In one method, it can be initially implanted as an unsculpted tube, then trimmed as necessary to preclude redundancy of the leaflets. This method can be performed as follows. The patient or animal is placed on total cardiopulmonary bypass so that the heart can opened safely. The heart is either arrested or fibrillated and the mitral valve is exposed through an incision in the left atrium. The leaflets and chordae tendineae of the native mitral valve are surgically removed, leaving behind a mitral valve annulus **121**. This annulus **121** has a roughly circular shape; however, as shown in FIG. **2**, there is a somewhat "flattened" area **123** in the annulus, on the side closest to the aortic valve. In an intact native mitral valve, the base of the anterior leaflet is attached to this "flattened" region of the mitral valve annulus. The inlet (proximal) **202** end of the tubular segment **200** is sutured into the mitral valve annulus **121**, using a suture line **204** which travels around the entire circumference of the annulus **121** and the tubular segment **200**. If desired, an annuloplasty ring (such as illustrated in FIG. **8**) can be used to create a bridge between the valve annulus **121** and the SIS tissue inlet **202**.

After the annulus has been properly secured, the length of the SIS segment can be trimmed to eliminate most of the excess length while retaining adequate tissue for the surgeon to work with. Sutures are then used to temporarily secure the outlet (distal) end of the tube to the papillary muscles at a distance from the mitral annulus compatible with the desired degree of "closure" of the tubular valve. This can be done by placing a tacking suture through the appropriate side of the tube distally and then passing it through the tip of the anterior papillary muscle **126**. The same procedure is performed on the opposite side of the tube, temporarily attaching it to the posterior papillary muscle **128**.

Saline is then injected into the left ventricular chamber **112**, which will remain capable of sustaining fluid pressure inside a closed chamber if access to the mitral valve is obtained via an incision through the left atrial wall. The saline flush generates fluid pressure in ventricle **112**, which causes the sides of the tube to be forced into approximation. In other words, the saline flush closes the newly-created valve.

Once the proper site of attachment of the distal end of the tube **200** to the two papillary muscles **126** and **128** has been determined, the tube **200** can be permanently attached to the tips of the papillary muscles **126** and **128**. This can be done in any of several ways. If the distal end of the SIS tissue has been carved, trimmed, or sculpted to create elongated wedges or strands of tissue which will serve as substitute chordae tendineae for attachment purposes, the distal ends of the sculpted tissue segments can be sutured to the papillary muscles. If desired, the tips of the tissue segments can be

inserted into small incisions in the tips of the papillary muscles; these can then be reinforced using reinforcing devices on the outsides of papillary muscles, to reduce the risk of tearing the tissue segments or the tips of the papillary muscles.

Alternately, preliminary tests on animals indicate that in animals which have normal papillary muscles of sufficient length, the distal end of the SIS tube does not need to be trimmed to create elongated wedges or strands of tissue for attachment purposes. If the blunt end of an SIS tube is sutured to the anterior and posterior papillary muscles, this will effectively create anterior and posterior leaflets that will emulate native leaflets after the heart starts beating again. Even if no tissue between the two leaflets is removed, it appears that a sufficiently large flow channel can be provided between the papillary muscles to handle the necessary flow.

Accordingly, it does not appear to be essential to remove such tissue by a sculpting or trimming procedure. Nevertheless, a sculpting or trimming step that physically divides the replacement leaflets into an anterior leaflet that has been partially divided from the posterior leaflet is presumed to be preferable, because it can help the valve leaflets more closely emulate the physical shape of the leaflets in a native mitral valve. If such a sculpting or trimming step is desired, it can be carried out at any suitable time during the operation, such as immediately after a temporary suturing step followed by a saline flush have confirmed that a certain attachment configuration will function in the desired manner.

After the SIS tissue has been permanently sutured to the papillary muscles, and after any trimming or sculpting step has been completed, the left atrium is closed and the heart is restarted. During each heartbeat, as blood crosses the valve in a forward direction, because of diastolic expansion of the ventricular chamber and contraction of the left atrium, the leaflets will be forced open.

It should be noted that this tubular valve, when open during the systolic phase of each heartbeat, has no obstructions in its flow path. By contrast, in every type of artificial mechanical valve being used today, various devices must be positioned in the flow path of the blood. These hinder blood flow, leading to pressure gradients across the valve, especially at high flow rates and with small sized valves, and they also generate turbulence that can damage blood cells. Moreover, since previous artificial tissue valves require stents and do not mimic the function of native valves, they also cause turbulence in blood flow, an important factor in the production of calcification in the leaflets of the artificial tissue valve.

As soon as ventricular expansion is complete and systolic contraction of the ventricle begins, the pressure generated inside the ventricle will press the two sides of the tube **200** against each other, closing the valve and forcing the blood to exit the ventricle through the aortic valve in the normal and proper manner. FIG. **3** shows a top view (from the left atrium) of the closed valve during contraction of the ventricle. The two sides of the tube effectively create a new anterior leaflet **122**A and a new posterior leaflet **124**A, which closely emulate the shapes and orientations of the native anterior leaflet **122** and the native posterior leaflet **124**. Closure (approximation) of the replacement leaflets **122**A and **124**A against each other, caused by the pressure of the blood inside the ventricle, prevents backflow of blood (regurgitation) back into the left atrium.

In a native mitral valve, the commissure between the two natural leaflets is curved, in a manner that resembles a smile. This occurs because: 1) the mitral valve annulus **121** is not completely round, and has a "flattened" region **123** on the side closest to the aortic valve, and 2) pressure on the outside of any tube that has one flat side and is attached at two points distally (in line with the two ends of the flat side) will result in apposition of the two sides of that tube in a manner that resembles a smile. In a simple tube, this results in the side of the tube that is flattened occupying the majority of the orifice. In the case of the mitral valve, it results in the anterior leaflet being larger than the posterior leaflet, thereby occupying the majority of the valve orifice in the closed position. As shown in FIG. **3**, this same type of "smile" commissure between the two leaflets is created by proper insertion and attachment of the replacement valve.

In some situations, it may be necessary to resect a small portion of the distal anterior wall of the SIS tube to prevent "systolic anterior motion" of the new anterior leaflet during systolic contraction of the ventricle. The phenomenon of systolic anterior motion of the anterior leaflet, which can lead to interference of blood flow through the aortic valve, is part of the etiology of a certain condition called Hypertrophic Obstructive Cardiomyopathy (HOCM), which affects some patients, and it must be avoided in patients who are likely to be subject to such a condition.

Tricuspid Valve Replacement

Tricuspid valve replacement in humans is especially problematic because tissue valves tend to fail earlier and mechanical valves tend to form clots at a much higher rate than either do in the mitral or aortic position. In addition, when tricuspid valves require replacement for infection, re-infection of the new artificial valve is so common that some authors have actually advocated removal of an infected native valve without replacement with a new artificial one! Thus, the need for a satisfactory artificial valve for tricuspid valve replacement is especially acute.

Tricuspid valve replacement can be created in essentially the same manner as mitral valve replacement, described above. Preliminary studies on animals indicate that the natural shape of the tricuspid annulus at the proximal end of a tube valve and three points of fixation distally to the normal papillary muscles in the right ventricle will result in an anatomically correct trileaflet tricuspid valve. However, it should be recognized that in the right ventricle, papillary muscles are more variable and unpredictable in their placement than in the left ventricle; accordingly, tricuspid valves tend to be more difficult to replace than any of the other three valves, and successful replacement depends even more heavily upon the experience and expertise of the surgeon in tricuspid valve repair than in other valves.

Optional Use of an Annuloplasty Ring for Replacement of Atrio-Ventricular Heart Valves.

A preferred mode for surgical insertion of the valves described herein completely avoids the use of an annuloplasty ring. As foreign objects that come into direct contact with blood (and which typically have rough surfaces, to facilitate sewing), annuloplasty rings in the mitral or tricuspid position pose a theoretical problem in regard to the threat of thrombosis. Accordingly, by disclosing replacement valves that can be created without using annuloplasty rings, this invention offers an advance over prior art replacement valves currently in use.

However, clinical practice has shown that any potential increase in the threat of thrombosis due to an annuloplasty ring in a mitral or tricuspid valve repair is moderately low; by way of illustration, surgeons often conclude that the increased risk of spontaneous hemorrhage associated with systemic anticoagulation outweighs the risk of thrombosis resulting from the use of annuloplasty rings. Accordingly,

annuloplasty rings are widely used without severe adverse effects, and in many patients suffering from heart disease or congenital abnormalities, they can be advantageous or even necessary.

Therefore, this invention discloses (1) replacement valves made of tubular tissue (or suitable synthetic material) coupled to annuloplasty rings, and (2) a method of surgically implanting such replacement valves. In such cases, the tubular tissue will work in the same manner as described above, while the annuloplasty ring will help create a bridge between the inlet end of the tubular tissue segment, and a valve annulus in a patient (such as a patient whose native mitral valve annulus is weak, dilated, and/or "rounded" out of its normal shape). The implantation of the annuloplasty ring can be carried out using the same techniques that are currently used for mitral and tricuspid valve reparative techniques. After a segment of SIS tissue has been harvested from the body of the patient receiving the heart valve, the inlet rim of the SIS tissue is sutured to an annuloplasty ring. After the annuloplasty ring has been sutured to an atrioventricular valve annulus, the distal end of the SIS tissue is sutured to the papillary muscles as described above.

Alternatively, if synthetic material or an SIS tissue segment from an animal or human cadaver is used, the annuloplasty ring can be coupled to the tube and both can be packaged together in a sealed package which maintains their sterility. This article of manufacture is described in more detail below and is shown in FIG. **8**, in which a tubular segment **500** has been attached to annuloplasty ring **502**. Accordingly, the subject invention discloses a method of using small intestinal submucosal (SIS) tissue in conjunction with an annuloplasty ring to create a heart valve replacement.

It should be noted that the tubular valves of this invention do not require stents. As used herein, the term "stent" includes any man-made device (other than a suture, annuloplasty ring, or leaflet material) which is surgically implanted in a patient's heart (or aorta or pulmonary artery) as part of a replacement valve, and which is contacted by blood which flows through the heart (or aorta or pulmonary artery). Stents are major components in all mechanical replacement valves, since they must securely hold the ball, flapper, or other movable elements of the valve in proper position; they are also used in nearly every type of artificial tissue valve, to secure the tissue flaps in the proper configuration. The term "stent" does not include reinforcing pledgets placed on the outside of an aorta or pulmonary artery, since such pledgets would not be contacted by blood flowing through the artery.

Stents are known to increase turbulence and thrombosis. Since the valves disclosed herein are stentless, this invention offers an important advance over prior art replacement valves which are currently approved by the FDA.

In summary, the steps for creating an atrioventricular (mitral or tricuspid) replacement valve can be described as follows:

1. A tubular segment is obtained, consisting of thin and flexible tubular tissue or synthetic material, having an inlet end and an outlet end.
2. The damaged or deformed leaflets of the native diseased valve are surgically removed from the heart of the patient, to generate an open valve annulus. The chordae tendineae are also removed, while the papillary muscles in the ventricular chamber are left intact.
3. The inlet end of the tube is sutured to the valve annulus, or to an annuloplasty ring if necessary for a specific patient.
4. The outlet end of the tube is sutured to the papillary muscles, in a manner which will allow the outlet flaps to function as valve leaflets that will open during ventricular diastole, when blood flows from the atrium into the ventricle. The valve leaflets will approximate and close the valve during ventricular systole, to prevent backflow when fluid pressure in the ventricle exceeds fluid pressure in the atrium. The specific sites of attachment of the tubular material to the papillary muscles should allow the resulting leaflets to approximate in a manner that emulates the shape and angular orientation of the leaflets in a properly functioning native valve.

Aortic (and Pulmonic) Valve Replacement

In a preferred method of creating a replacement for a semilunar valve (i.e., an aortic or pulmonary valve), a segment of intestinal tissue several inches long is removed from the patient and treated to remove the serosa, smooth muscle, and mucosal layers in the same manner described above. This leaves a tubular structure made of the basement membrane and submucosal layers, referred to herein as small intestinal submucosal (SIS) tissue. Alternately, as with atrioventricular tubular valves, the tubular material may be obtained from other animals or from human cadavers, or it may be manufactured from a suitable synthetic material. For convenience, the discussion below will assume that an SIS segment is used. The desired length can range from about 2 cm for neonates to about 6 cm for adults.

To secure a tubular segment **200** inside an aorta in accordance with one method (the same approach can be is used to create a pulmonic valve in a pulmonary artery), an aortic wall is opened by an incision above the level of the commissural posts of the aortic valve, and the cusps of the native aortic valve are removed, leaving behind a valve annulus. The tubular SIS segment **200** is then inserted, and as shown in FIGS. **4** and **5**, the tubular segment **200** includes an apparatus for attaching the inlet end to the interior surface of the aortic wall **250**. The apparatus for attaching the inlet end comprises an inlet attachment portion **203** on the tubular segment **200** that extends around the circumference of the tubular segment. The inlet attachment portion **203** on the tubular segment **200** is secured to the interior surface of the aortic wall **250** by a circumferential suture line **210**. This step can utilize an annuloplasty ring if desired. The tubular SIS segment **200** also includes an apparatus for attaching the outlet end of the tubular segment directly to the interior surface of the aortic wall **250**. The apparatus for attaching the outlet end comprises first **223**, second **224**, and third **225** attachment points on the tubular segment **200**. Each attachment point is secured to the aortic wall **250** by attaching sutures **200**, including first **226**, second **227** and third **228** attaching sutures. In one preferred embodiment shown in FIG. **4**, the attaching sutures **220** are shown to comprise three longitudinal suture lines **220** spaced at one-third intervals (120° apart from each other) around the internal periphery of the aortic wall **250**.

Suturing the tissue segment **200** to the inside of the aortic (or pulmonary artery) wall **250** by the three longitudinal suture lines forms an arrangement for opening and closing the outlet end **204** of the tubular segment. The arrangement for opening and closing the outlet end of the tubular segment includes three flexible tissue regions **222**, namely first **230**, second **231** and third **232** flexible tissue regions. The flexible tissue regions **230**, **231**, **232** function as respective first **233**, second **234** and third **235** valve cusps during operation of the valve, as shown in FIG. **5**. After the tissue segment **200** is properly secured and the patient's heart is closed by the

surgeons and restarted, the three valve cusps **233–235** will go through a cyclical movement with each heartbeat. During the systolic stage (ventricular contraction) of each heartbeat, depicted in FIG. **4**, the cusps **222** be held in an open position by blood entering the inlet end **202** and exiting the outlet end **204**. When the systolic stage ends and the left ventricle begins to expand during diastole, back pressure in the aorta (or pulmonary artery) causes the three valve cusps **233–235** to flex in a downward and inward direction. However, the cusps are constrained and their motion is limited by the attaching sutures **220**. The combination of pressure and tension causes the three valve cusps **233–235** to flex inwardly, as shown in FIG. **5**, thereby forcing the valve into a closed position and preventing backflow into the ventricle.

An alternative to placing the three parallel rows of suture lines inside the aorta (or pulmonary artery) as described above is to fix the outlet end of the tube valve at three equidistant points (120° apart around the circumference of the outlet end) only. This technique will preclude the necessity for the longitudinal suture lines described above but will allow the valve to function in the same manner.

If desired, the three longitudinal suture lines **220** (or the three points of fixation of the outlet end of the tube) can be reinforced by strips (often called pledgets) placed on the exterior of the aortic wall. These reinforcing strips can be made of autologous tissue, materials sold under trademarks such as TEFLON, GORETEX, SILASTIC, or any other suitable material. Since these strips would be positioned outside the aorta or pulmonary artery, they would not come into contact with blood flowing through the artery. Therefore, they can reinforce the arterial wall, distribute any tensile stresses more evenly across a wider area of the arterial wall, and reduce the risk of tearing the arterial wall, without increasing the risk of thrombosis inside the artery. Depending on the positioning of the replacement valve in the aorta, it may also be desirable to place a similar strip around the exterior of an aorta or pulmonary artery to reinforce the circumferential inlet suture.

If a need becomes apparent in a specific patient, similar reinforcing strips can also be positioned inside an aorta or pulmonary artery, and a stent can be used to reinforce the inlet attachment portion. However, any reinforcing component which is exposed to blood inside the artery would increase the risk of thrombosis and probably would suggest to the surgeon that the patient would need to be placed on anticoagulant drugs to reduce the risk of clot formation.

In some patients, it may be preferable to use an annuloplasty ring for replacement of an aortic or pulmonary valve. Accordingly, the subject invention discloses a method of replacing the aortic and pulmonary valves in which a round annuloplasty ring is used in conjunction with the artificial tubular tissue or mechanical valve. After obtaining a tubular segment of tissue or synthetic material, the inlet attachment portion of the tubular segment is sutured to a round annuloplasty ring which is then sutured into the aorta (or pulmonary artery) at the level of the lowest point of the excised native semilunar valve. The distal end of the tubular segment for both aortic valves and pulmonary valves is then handled in the same manner as described above for these valves without annuloplasty rings.

Two additional variations in aortic and pulmonary replacement valves have been recognized and will be evaluated if an apparent need arises. First, initial tests on dogs, coupled with computer analysis using an iterative finite-element algorithm to calculate the stresses on each portion of a cylindrical tissue segment constrained as described herein, have indicated that satisfactory results are obtained if the outlet end of the tissue cylinder is cut in a planar manner, perpendicular to the main axis of the cylinder. This can be regarded as a blunt-end or square-end cut. As an alternative method of sculpting the tissue segment, non-planar cuts (such as a mildly sinusoidal cut) can be used to generate three flaps of tissue that extend slightly beyond the outlet ends of the longitudinal suture lines (or fixation points) or to slightly scallop the outlet end of the tube valve, as is more characteristic of the native semilunar valves. Non-planar outlets have not yet been evaluated, but they can be tested using any of several techniques (computerized CAD-CAM analysis, in vitro testing using a closed mechanical pumping circuit, or in vivo using animals such as dogs or sheep) to determine whether they are preferable to a square-end outlet, either for particular patients or as a general. approach.

In summary, the steps for creating a semilunar replacement valve (i.e., an aortic or pulmonary valve) can be described as follows:

1. A tubular segment is obtained, consisting of thin and flexible tissue or synthetic material having an inlet end and an outlet end.
2. The damaged or deformed leaflets of the native valve are surgically removed, to generate an open valve annulus.
3. The inlet end of the tube (or an incorporated annuloplasty ring) is sutured to the valve annulus.
4. The outlet end of the tube is sutured to the aorta or pulmonary artery at three equidistant points around the circumference. This creates three flexible tissue regions between the three points of attachment, and the flexible tissue regions will function as valve cusps that will open during ventricular systole, when blood flows from the ventricle into the aorta or pulmonary artery. The valve cusps will approximate and close the valve during ventricular diastole, to prevent backflow when fluid pressure in the aorta or pulmonary artery exceeds fluid pressure in the respective ventricle.

Based on the information available to date, including animal tests as well as computer simulations and the Applicant's extensive experience in cardiac surgery, it appears that it is not necessary to provide any additional safeguards to ensure that the three valve cusps in a replacement aortic or pulmonary valve come together and close during each diastolic cycle, rather than being flattened against the inside of an aortic wall (or pulmonary artery wall). Nevertheless, it is recognized that if the back pressure in the aorta were to flatten any of the three valve cusps against the artery wall, rather than causing all three to close together, closure of the valve would be prevented and regurgitation (i.e., reentry of the blood into the ventricle) would result. Accordingly, if it is desired to increase the level of assurance that flattening of the cusps against the interior wall of the artery will not occur during diastole, either as a general precaution or in patients having certain abnormal conditions, then at least two methods are available to reduce such risks.

The first method involves creating a partial closure of adjacent cusps at their outer periphery. This can be done by gently pinching the walls of the inserted SIS cylinder **200** together at the outlet end of each of the three longitudinal suture lines **220** (or outlet attachment points), as shown in FIG. **6**. The pinched SIS junctures can then be held in place by one or more suture stitches **240**. If desired, the suture stitches **240** can be reinforced to prevent tearing of the SIS segment **200** by placing small reinforcing pieces **242**, made of a flexible, soft, blood-compatible material such as Gore-Tex or Silastic, on the outside surfaces of the SIS wall **200**.

As shown in FIG. 6, the pinched junctures include first **243**, second **244** and third **245** pinched junctures which can be held in place, along with reinforcing pieces **242**, by the suture stitches **240**.

An alternate potential method for ensuring that the three cusps will not become flattened against the inside of the aorta (or pulmonary artery) involves a stent device that could be secured within the aortic wall **250**, outside the SIS segment **200**. This type of stent has projections which extend in an inward radial direction, toward the central axis of the aorta. These projections, positioned at midpoints between the three attachment points at the outlet end, prevent any flattening of the cusp regions **222** against the interior of aortic wall **250**. This ensures that back pressure in the aorta forces each cusp in an inward direction, to ensure closure, rather than pressing the cusps in an outward direction which could cause them to flatten against the interior of the arterial wall and allow regurgitation.

The use of such a stent probably requires placing the patient on anticoagulant drugs to reduce the risk of thrombosis. Nevertheless, the blood is not forced to flow through any mechanical elements as are currently used in conventional caged-ball, bi-leaflet, or tilting disk valves; instead, the blood flows through a cusp arrangement which uses soft, flexible cusps. Therefore, this approach, even though it requires a stent outside the cusps to ensure closure, provides a valve that is less thrombogenic and less hemolytic than any currently available mechanical valves.

Reduction of Turbulence and Calcification by Tubular Valves

In addition to the various problems (particularly lack of durability) that are characteristic of conventional tissue valves in use today, it also appears that their designs may aggravate the problem of calcification, a major pathologic form of deterioration which leads to the failure of many presently available artificial tissue valves. Previous analyses regarding the etiology of calcification of artificial tissue valves have centered around (1) the tissues used to construct the valves, which presently are either porcine valve cusps or bovine pericardial tissue; (2) chemical fixation processes which are necessary to render heterograft tissues non-antigenic, or (3) non-chemical fixation processes, usually involving freezing, which are necessary to treat homograft tissues to reduce their antigenicity.

However, a highly important piece of evidence indicates that another factor is etiologically significant in tissue valve calcification, namely, the turbulence of blood flow that occurs within and around all artificial tissue valves constructed using prior art designs. Evidence that turbulence can cause or severely increase the risk of valve calcification in the absence of foreign material, fixation techniques, and antigenicity, is provided by the fact that over half of the patients who must undergo surgery for calcific aortic stenosis were born with a bi-leaflet aortic valve, a condition which is notorious for causing turbulent flow. In these patients, neither antigenicity nor fixation processes can be incriminated as causes of valve calcification, since the patient's own valve is the one that has calcified. Therefore, the high rates of calcification encountered in abnormal bi-leaflet aortic valves offers strong evidence that turbulent blood flow, per se, can cause or severely increase the risk of calcification of valves.

Preliminary studies suggest that by reproducing the manner in which native valves function, less turbulence will be generated as blood passes through the valves disclosed herein, compared to conventional replacement valves. Therefore, it appears likely that this reduction in turbulence will, in turn, reduce the likelihood that the tubular tissue valves described herein will calcify.

Use of Intestinal Tissue in Heart Valves

To the best of the Applicant's knowledge, it has never previously been disclosed or suggested that autologous human intestinal tissue, specifically the submucosa of the small intestine (SIS), can or should be used to create all or part of a replacement artificial heart valve in a patient with a defective or diseased heart valve. Since autologous intestinal tissue, when harvested and treated as described above, appears to be very well suited to this use, and since it offers a number of important advantages over materials used in conventional heart valve replacements (including the complete absence of antigenicity, and the absence of the requirement of chemical fixation of the tissue prior to implantation), an important aspect of this invention is the disclosure, in broad terms, that intestinal tissue harvested from the body of the same patient who is receiving a new heart valve can be used in the replacement valve.

Accordingly, this invention discloses a method of surgically replacing a heart valve in a human patient in need thereof, comprising the steps of (a) extracting a segment of intestinal tissue from the patient's abdomen, and (b) using the intestinal tissue to form at least one component of a replacement valve for the patient's heart. It also discloses certain articles of manufacture comprising previously prepared intestinal segments, from animals or human cadavers, which have been treated to render them suitable for use in creating replacement valves, and which are contained in sealed packages that maintain their sterility. These articles of manufacture are discussed in more detail below.

Other Tissue Sources

Although autologous SIS intestinal tissue described above appears to be an ideal tissue for creation of artificial tissue valves, the critical factor in the construction of such artificial tissue valves remains the tubular shape of the tissue or material to be implanted rather than the specific source of origin of that tissue or material.

Various other types of tissue from the body of the patient receiving the heart valve replacement can be used if desired, rather than intestinal tissue. For example, in most patients, the pericardial sac which encloses the heart has enough tissue so that a segment can be removed and used as a heart valve. This would allow a surgeon to conduct the entire operation without having to make an additional incision in the patient's abdomen. In fact, recent studies by others have indicated the feasibility of using freshly harvested autologous pericardial tissue to create artificial cusps that can then be sutured inside the aorta to serve as an artificial aortic valve. That technique, however, differs in several ways from the current invention, and those investigators apparently have not recognized the importance of the principle that Form Follows Function. Their technique is designed to create artificial cusps that look like the native aortic valve cusps from fresh autologous pericardium in hopes that they will function like the native cusps. In other words, their apparent goal and principle is to force function to follow form. By contrast, the subject invention states that pericardial tissue (which is essentially flat) can be used to replace an aortic valve if desired, but the pericardium should first be fashioned into a tube, and that tube should be fixed inside the aorta in the manner described above. By fixing the inlet end of the tube circumferentially and the outlet end of the tube at three (3) points (or along three longitudinal lines from the inlet), the external diastolic pressure in the aorta will cause the non-fixed sides of the tube to collapse against one another and the pericardial tube will be forced into the shape

of a normal aortic valve. In other words, "Form Follows Function". The principle that Form Follows Function will be operative in all artificial tubular valves used to replace any of the four native valves regardless of the specific type of tissue used to create the tubes.

In view of encouraging results obtained to date with intestinal tissue, and in view of the abundant supply of small intestinal tissue in all patients, other types of autologous tissue have not been evaluated to determine whether they are sufficiently durable and flexible for use as a heart valve. However, if the need arises, other types of autologous tissue can be evaluated using routine experimentation. For example, a potential source of tissue is the "fascia lata," a membranous layer which lies on the surface of certain skeletal muscles.

Another potential source of autologous tissue is suggested by a known phenomenon involving mechanical objects that are implanted in the body, such as heart pacemakers. When such objects remain in the body for several months, they become encapsulated by a layer of smooth, rather homogeneous tissue. This phenomenon is described in articles such as Jansen et al 1989. The cellular growth process can also be controlled by manipulating the surface characteristics of the implanted device; see Chehroudi et al 1990. Based upon those observations and research, it is possible that mandrel implantation in the body of a patient who will need a heart valve replacement might become a potentially feasible technique for generating the cylindrical tissue.

As another potential approach, it may be possible to generate unlimited quantities of cohesive tubular tissue segments with varying diameters, for use in patients of different size, using in vitro tissue culture techniques. For example, extensive work has been done to develop skin replacements for burn victims and tubular vascular grafts, by seeding viable connective tissue cells into lattices made of collagen fibers. Collagen is the primary protein that holds together mammalian connective tissue, and the lattice provides the cells with an environment that closely emulates the environment of natural tissue. The cells will. grow to confluence, thereby forming cohesive tissue, and some types of cells will secrete enzymes that gradually digest the artificial collagen matrix and replace it with newly generated collagen fibers secreted by the cells, using the natural process of collagen turnover and replacement. This type of cohesive tissue culture is described in articles such as Yannas et al 1989 and Tompkins and Burke 1992.

Either of these approaches (mandrel implantation or ex vivo tissue culturing) would require careful evaluation to determine whether the resulting tissue would be suitable for long-term use in heart valves. With the promising results obtained to date with intestinal tissue, which is in abundant supply, there does not seem to be an apparent need to undertake such tests at the present time.

In an alternate embodiment of the subject invention, "homograft" tissue is harvested from the bodies of human cadavers for later use in artificial tubular heart valves. For example, a very long segment of intestinal tissue comprising all or a major portion of the jejunal region of the small intestine is resected from the body of someone who has recently died, such as an accident victim. This harvesting operation would be comparable to harvesting a heart, kidney, or other internal organ from a deceased organ donor. The intestinal tissue is then cut into segments of roughly 10 to 20 cm (four to eight inches) each, which is prepared (by removing the serosa, smooth muscle, and submucosal layers), treated to reduce its antigenicity, and stored (at either refrigerated or frozen temperature) in a sterile preservation solution until use. When needed as a heart valve replacement, the tissue is warmed and treated as necessary, and cut into the precise size and configuration needed.

One advantage of this approach is that it spares the cardiac patient from any additional pain or surgical stress that might result from having a surgical incision made in the abdomen to harvest autologous SIS tissue as described above. However, the additional stress or pain of obtaining a segment of intestinal tissue through a small abdominal incision is quite small compared to open-heart surgery, where the chest and rib cage must be opened. Indeed, several of the newest approaches to coronary artery bypass surgery (the most frequently performed cardiac operation) require much larger abdominal incisions to harvest abdominal arteries that are now used as bypass conduits.

Another alternate embodiment is to use "heterograft" tissue from other animal species. This embodiment probably requires chemical fixation of the heterograft tissue (which presumably would comprise intestinal segments) by techniques such as glutaraldehyde crosslinking, as currently used to fix porcine or bovine pericardial tissue for conventional heart valve replacements. Although one might expect intestine-derived tubular tissue fixed in glutaraldehyde to have problems similar to the presently available tissue valves, the calcification and durability problems of current tissue valves should be substantially reduced because of the tubular structure of the resultant valves, which would reproduce the function of the native valves, thereby leading to less turbulence and hence, less calcification, and greater long-term durability. It should also be noted that researchers are creating, using breeding as well as genetic engineering techniques, various strains of animals (mainly pigs) that have reduced tissue antigenicity (see, e.g., Rosengard et al 1992 and Emery et al 1992). Such animals may be able to provide tissue which needs minimal fixation, or possibly no fixation treatment at all.

Tubular "Mechanical" (Non-Tissue) Valves

In addition to providing a method of using tubular human or animal tissue to create replacement valves, this invention also suggests the use of tubular synthetic material as a starting material for such valves. Various types of highly durable and flexible synthetic materials have been developed and are continuing to be developed, and some of these materials are promising candidates which can be evaluated for possible use as described herein. One such material is sold under the trademark "GoreTex." It is, in essence, a polymerized layer of PTFE which is rendered flexible by coating it onto a flexible woven or knitted substrate material, such as nylon fabric. By coating PTFE onto a tubular substrate, it is possible to create tubular forms of such coated materials. Although such materials are highly durable inside the body, they can occasionally causes problems of blood clotting, apparently due in part to their rough surface textures, and possibly due also to plasticizers and other chemicals used to control the polymerization, thickness, and flexibility of the PTFE coating material.

Perfluorinated elastomers, a different class of synthetic materials that have recently been developed, also offer promise as potential artificial tubular valves as described herein. These elastomers are described in patents such as U.S. Pat. No. 4,900,793 (Lagow and Dumitru, 1990). Essentially, they contain only carbon and fluorine atoms, which are bonded together in highly stable polymeric configurations. Perfluorinated elastomers contain very little oxygen, hydrogen, nitrogen, sulfur, or other substances that might chemically react with physiological fluids to degrade the elastomer or cause leaching of constituent ions into the

blood. Tbese elastomers can provide very smooth surfaces, and since they are elastomeric in their own right, it is unnecessary to coat them onto tbe rough surface of a second material such as woven or knitted nylon in order to provide flexibility. They can be molded or otherwise synthesized directly into tubular form.

An additional advantage that can be obtained by using synthetic materials in the manner disclosed herein is that an essentially tubular configuration can be provided which has a gradually varying diameter. For example, a relatively long tubular device can be created from synthetic material, having a diameter at tbe inlet end of up to about 5 cm and a diameter at the outlet end of about 2 cm. A surgeon can simply cut the piece of tubing at any appropriate location along its length, to provide an inlet diameter corresponding to the diameter of a patient's valve annulus, which can be measured after tbe beart has been opened and the damaged or defective leaflets have been removed. In this manner, a single tubing size can be adapted to accommodate various different patients; this will reduce the costs that would be required to manufacture or stock tubes having multiple different sizes.

In the case of artificial "mechanical" (non-tissue) tubular valves, the more physiologic flow patterns should result in less, thrombogenicity and less turbulence, which are major problems witb presently available mechanical valves. The design disclosed herein is, to the best of the Applicant's knowledge, tbe only mechanical (non-tissue) valve design ever proposed that bas absolutely no obstructing part within the flow orifice of the valve in tbe open position. Conventional mechanical valves require hinge mechanisms, moving discs, large struts, caged balls, or bulky sewing rings, all of wbieb have been incriminated as etiologic factors in the inberent thrombogenicity and/or sub-optimal hemodynamics of previously constructed mecbanical heart valves, especially tbose of smaller sizes. Even tbe McGoon and Roe-Moore valve designs (described as "extinct" in Bodnar and Frater 1991, pp. 319–321) required obstructions in the flow path; those valves returned to a closed position when at rest, and tbe leaflets wbicb blocked the flow path had to be forced opened in order for blood to flow through those valves. By contrast, the tubular valves disclosed herein are effectively open wben at rest, and tbe atrioventricular leaflets or semilunar cusps close only wben tbey are forced into a closed position by blood pressure. Compared to all previously available or proposed mecbanical valves, the mechanical valves disclosed herein will bave better hemodynamic characteristics and are likely to be less thrombogenic.

Finally, although the durability of conventional mechanical valves is considered to be their most attractive feature, valve failures do occur. These structural failures are invariably due to high mecbanical stresses and/or trauma tbat are focused on certain points in a given valve design. Such repetitive, focused stresses can eventually result in the failure of the materials used to construct such valves. By contrast, the computerized analytical studies on tubular valves, described above, indicated that the distribution of stress in a tubular replacement valve as described herein is virtually identical to tbe distribution of stress in native heart valves; such natural stress distributions can be assumed to be optimal. Furtbermore, the areas of maximal stress encountered by tubular replacement valves were relatively low in magnitude, since they were distributed over larger surface areas, when compared to conventional mechanical valve designs. Therefore, the fact that tubular replacement valves are stressed in an apparently optimal fasbion, as dictated by nature, indicates that they will have less risks of stress-related mechanical failure than conventional mechanical valves.

Articles of Manufacture

In addition to disclosing a metbod of surgery, this invention discloses an article of manufacture depicted in FIG. **7**. This item comprises a tubular segment **500**, made of tissue from the small intestine, or synthetic material having suitable overall dimensions and walls sufficiently thin and flexible to allow it to function as leaflets in a heart replacement valve. Since autologous tissue would not require storage for later use, this portion of the disclosure relates to the use of non-autologous (homograft or heterograft) tissue. This tubular segment **500** is enclosed within a sealed container **510** that maintains sterility of the segment **500**. Such a sterile container **510** can comprise a plastic pouch, as shown in FIG. **7**, baving a transparent front layer **512** to allow visual inspection (tbis layer is shown folded up at one corner, for depiction purposes only). The front layer **512** is sealed around its periphery to a back layer **514**.

In an alternate article of manufacture, depicted in FIG. **8**, the tubular segment **500** is attached to an annuloplasty ring **502** before both are sealed inside package **510**. The tube-to-ring attachment can be done by suturing in tbe case of tubular tissue segments, or by any suitable synthetic method (such as molding) if a synthetic tube is attacbed to a synthetic annuloplasty ring.

If intestinal tissue is used, it should be packaged in hydrated form, in a suitable liquid (such as phosphate-buffered saline witb one or more preservative agents, such as glutaraldehyde, if desired) contained witbin the pouch. Such pouches can be sbipped and stored in stiff-walled boxes for protection; alternately, instead of using a flexible pouch as the container, a plastic box with a transparent elosure layer sealed around its rim can be used as the container itself.

If a segment of intestinal tissue is contained in such a sterile container, it preferably should be treated before being sealed within the pouch, to remove the exterior serosa and muscularis layers and the interior mucosal layer, leaving only the desirable submucosal and basement layers.

In another preferred embodiment involving synthetic materials, a synthetic tube as described herein can bave a diameter that varies gradually over its length. Such a tube can be transsected at a location having the desired diameter. This would allow a tube with a single size to accommodate patients who have valve annulus diameters witb varying sizes.

In another preferred embodiment involving synthetic materials, different sizes of synthetic tubes can be packaged separately. That raises an important issue. In most cases, it will probably be possible to directly insert an SIS intestinal segment harvested from a patient directly into the mitral or tricuspid valve location in that patient, since the diameters of an SIS segment and a mitral or tricuspid valve annulus in most humans is believed to be comparable or at least compatible. However, this does not appear top be the case with aortic or pulmonary valve replacements; in most patients, the diameter of an SIS segment is likely to be too large for direct insertion in tubular form into the aorta or pulmonary artery of that patient. To cope with this problem, an SIS segment can be cut longitudinally, to convert it into a flat segment that can be trimmed to any desired size before creation of the replacement valve. As described above, three longitudinal suture lines can be used to create a replacement aortic or pulmonary valve, as part of the surgical procedure. Therefore, one of these suture lines can be used to re-size the SIS segment into a different diameter while retaining its tubular form.

Alternately, to replace any of the four heart valves, a prepackaged intestinal tissue segment can be used which has

the desired diameter, harvested from a human cadaver or animal. This would avoid the need for a longitudinal cut, and it would preserve the tubular form of the intestinal segment throughout the entire procedure. Tissue from cadavers or animals would need to be treated by fixation (using glutaraldehyde crosslinking or another suitable method) to reduce its antigenicity before implantation.

Accordingly, one of the preferred embodiments of this invention comprises an array of sealed sterile packages containing intestinal tissue segments from cadavers or animals for use in creating replacement valves. Each separate container would hold an intestinal segment having a known diameter or circumference (presumably measured in millimeters), which would be indicated on the label of the container. Before being packaged, the tissue segments would be cleaned to remove the undesired tissue layers and fixed to reduce antigenicity. After a surgeon opens a patient's chest and excises the cusps or leaflets from a diseased or damaged valve, he or she can directly measure the valve annulus. This measurement will indicate the exact diameter or circumference of the intestinal segment that should be used. A sealed package containing an intestinal tissue segment having the desired diameter or circumference can be selected and opened, and the segment will be immediately available. If desired, the tissue segment in the sterile package can already be attached to an annuloplasty ring, which would also be contained in the package.

Synthetic material can be manufactured in tubular form by various means, including extrusion, and coating (either externally or internally) of a liquid resin, monomer, or other fluid onto a cylindrical mold, followed by curing (using heat, chemicals, ultraviolet radiation, etc.) of the fluid into a solidified film. The synthetic tube can be packaged in a sterile liquid if desired, to avoid any possibility of dehydration, cracking, flattening under pressure (which might cause formation of seams), or other degradation.

Thus, there has been shown and described a new and useful article of manufacture and method for create replacement heart valves from tubular tissue or synthetic material. Although this invention has been exemplified for purposes of illustration and description by reference to certain specific embodiments, it will be apparent to those skilled in the art that various modifications and alterations of the illustrated examples are possible. Any such changes which derive directly from the teachings herein, and which do not depart from the spirit and scope of the invention, are deemed to be covered by this invention.

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What is claimed is:

1. A prosthetic heart valve, comprising:

at least two cusps comprised of a thin and flexible material, each cusp having an inner surface and an outer surface and each cusp being attached to another cusp on both sides of the cusps along longitudinal suture lines, each of the cusps adapted to flex inwardly into and out of engagement with another cusp so as to close and open the valve in response to force by blood pressure;

wherein a portion of the inner surface of one cusp is in a facing relationship with a portion of the inner surface of another cusp adjacent the longitudinal suture line when the valve is open so that the cusps are biased partially closed even when the valve is generally open.

2. The heart valve of claim 1, wherein the valve has an inlet end and an outlet end, and a portion of the inner surface of each cusp is in a facing relationship with a portion of the inner surface of each adjacent cusp adjacent the longitudinal suture line and at the outlet end of the valve.

3. The heart valve of claim 1, wherein the thin and flexible material comprises pericardial tissue.

4. The heart valve of claim 1, wherein at least one suture holds a portion of the inner surface of adjacent cusps together.

5. The heart valve of claim 4, wherein the inner surface of adjacent cusps are held together toward the out-flow end of the valve so that the cusps are biased partially closed even when the valve is generally open.

6. The heart valve of claim 4 additionally comprising suture reinforcements.

7. The heart valve of claim 1 wherein reinforcing pieces holds a portion of the inner surface of adjacent cusps together.

* * * * *

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United States Patent [19]

Love

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[54] **STENTLESS HEART VALVE**

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[21] Appl. No.: **170,002**

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[52] **U.S. Cl.** **623/2**; 623/900

[58] **Field of Search** 623/2, 66, 900

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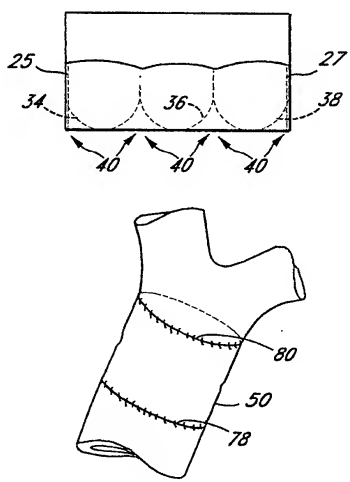
[57] **ABSTRACT**

A stentless heart valve assembled from autologous tissue is provided. The tissue is cut into a rectangular shape, folded, and stapled at several locations to form the cusps of the valve. The outer walls of the valve are pinched at the midpoint of each cusp to prevent the cusps from adhering to the valve walls. The ends of the rectangle are then stapled to form the annular valve itself.

30 Claims, 3 Drawing Sheets



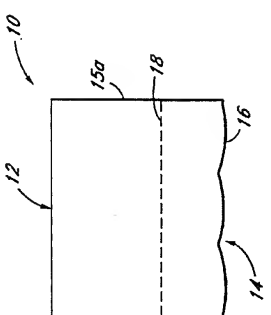


FIG. 1

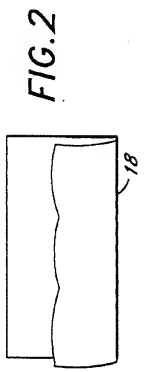


FIG. 2

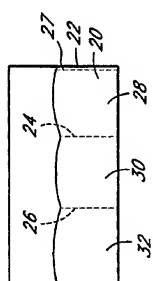


FIG. 3

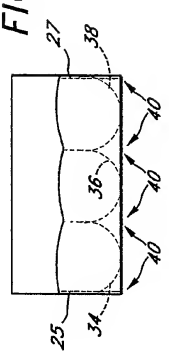


FIG. 4

FIG. 5

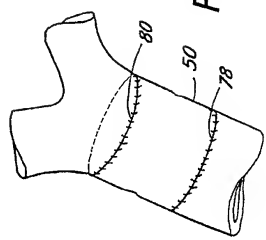


FIG. 6

FIG. 7

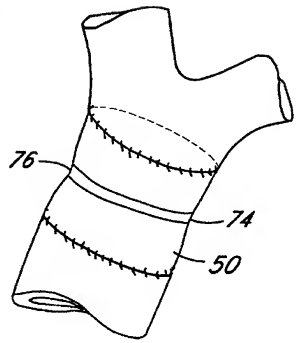
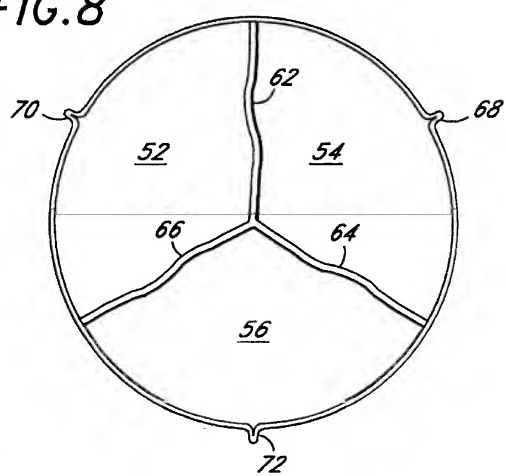


FIG. 8



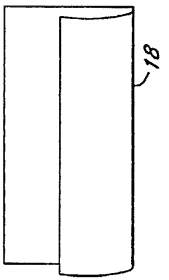


FIG. 10

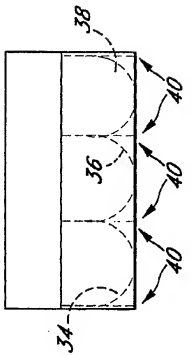


FIG. 12

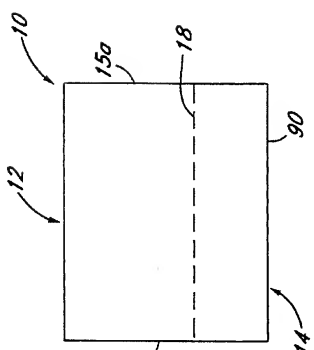


FIG. 9

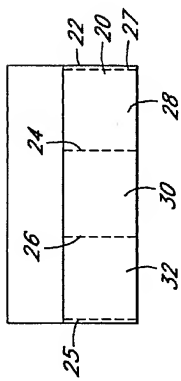


FIG. 11

STENTLESS HEART VALVE

BACKGROUND OF THE INVENTION

The present invention relates to heart valve replacements. Replacements are required for patients whose own valves have begun to fail. While many heart valve replacements rely on combinations of tissue and mechanical elements, the creation of valves from tissue, including autologous tissue, without the use of a supporting assembly or stent is also known. Senning, for example, describes such a technique for replacing a diseased heart valve with one made from the patient's own tissue in the Journal of Thoracic and Cardiovascular Surgery at Vol. 54, p. 465–470 (1967). Senning employed a piece of the patient's untreated fascia lata to fashion a trileaflet valve and sutured the new valve to the patient's native valve remnant with a continuous suture, reinforced at the valve's commissures with pledgeted sutures.

While Senning and other surgeons recorded some notable successes with their stentless autologous-tissue heart valves, they quickly encountered several difficulties. These included shrinkage and calcification of the tissue comprising the replacement valve. Additionally, fabrication of autologous-tissue valves during cardiac surgery required great skill and could not be done rapidly. Consequently, the technique of using autologous tissue to fashion stentless heart valves was soon abandoned by most surgeons in favor of the use of glutaraldehyde-tanned, stent-mounted valves, or mechanical prostheses.

Exemplary embodiments of such bioprostheses are disclosed and claimed in U.S. Pat. No. 4,470,157 and 5,163,955 assigned to Autogenics, assignee of this application, which uses stent assemblies to support a piece of autologous tissue.

SUMMARY OF THE INVENTION

The heart valve of the present invention is a self-supporting stentless prosthetic heart valve preferably formed during open heart surgery from autologous pericardium, removed from the patient during the surgical procedure. The only operations required to construct the heart valve are cutting to a precise geometry, folding, and securing the tissue together with staples or other fastening means. During surgery, the patient's diseased valve is excised and the annulus of the valve is measured. The tissue used to construct the valve is then cut from the patient with the aid of a template.

The heart valve replacement of the present invention is constructed from a single piece of autologous tissue treated by brief immersion in a weak glutaraldehyde solution. Brief immersion of the tissue aids in preventing calcification of the valve after implantation. After brief immersion, the tissue is cut into the required shape, preferably by use of a size-specific cutting die. Size-specific components, such as the cutting die, are preferably provided in kit form. The provision of kits corresponding to each annulus size advantageously puts all the items required by the surgeon to perform the valve replacement within ready reach, thereby minimizing the time required for the surgery.

The valve itself is a self-supporting annular body of tissue having distal and proximal ends and end edges. The proximal end of the tissue is preferably cut into a plurality of scalloped portions, and the tissue is folded to form inner and outer layers or walls of tissue. The distal portion located above the top or distal end of the inner layer of tissue serves as an outflow duct, and can be tailored to fit more closely the individual patient's vascular anatomy.

The inner and outer layers of tissue are secured together by a first set of staples or other suitable fastening means along a plurality of lines extending distally from the folded, proximal end of the tissue. This first set of staples forms a series of segments or pockets which form the leaflets of the valve. Next, the layers are again secured together by a second set of staples or other fastening means along the bottom of each of the segments in a series of arcs tangent at their midpoints to the fold. This second set of staples advantageously removes areas of bloodflow stagnation which would otherwise form during operation of the valve in the corners of each of the segments or pockets.

The valve maker then staples or otherwise secures the end edges of the tissue together to form the cylindrical body of the valve. The inner layers of tissue in each of the segments or pockets form each of the cusps of the completed valve. These cusps coapt with each other along three lines extending radially inward from the annulus of the valve.

The present invention advantageously prevents the cusps of the valve from adhering to the outer layer of tissue, by providing a staple or suture emplaced in the outer wall of the valve at a location midway along each segment wall opposite the distal end of the inner layer of tissue. In an alternative embodiment of the invention, this objective is achieved by securing a girdle around the midsection of the valve to form a sinus.

The stentless or self-supporting valve of the present invention is therefore quickly and easily, repeatably and accurately fabricated. The use of standardized, size-specific kits and a precise assembly technique advantageously allows the valve to be precisely fabricated in a matter of minutes during the open heart surgery procedure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a top view of a section of tissue used to construct the heart valve of the present invention.

FIG. **2** is a top view of the tissue section depicted in FIG. **1** after it has been folded.

FIG. **3** is a perspective view of the tissue section depicted in FIG. **2** illustrating the placement of the first longitudinal rows of staples.

FIG. **4** is a top view of the tissue section depicted in FIG. **3** illustrating the placement of the second row of staples in curvilinear geometry.

FIG. **5** is a perspective view of the valve constructed from the tissue section illustrated in FIG. **4**.

FIG. **6** illustrates the implantation of a valve into the heart of a patient in the pulmonary outflow tract.

FIG. **7** is a schematic view of an implanted embodiment of the heart valve of the present invention, illustrating the placement of a girdle around the valve.

FIG. **8** is an elevation view of the valve leaflets of the present invention when in the closed position.

FIG. **9** is a top view of a section of tissue used to construct a second embodiment of the heart valve of the present invention.

FIG. **10** is a top view of the tissue section depicted in FIG. **9** after it has been folded.

FIG. **11** is a perspective view of the tissue section depicted in FIG. **10** illustrating the placement of the first row of staples in an alternate embodiment of the present invention.

FIG. **12** is a top view of the tissue section depicted in FIG. **11** illustrating the placement of the second row of staples in curvilinear geometry.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The self-supporting heart valve of the present invention is constructed from a single piece of tissue without the need for a supporting stent. The heart valve of the preferred embodiment of the present invention has three leaflets, formed from the piece of tissue and coapting between three commissures. The only operations required to construct the heart valve are cutting to precise geometry, folding, and securing the tissue together with staples or other fastening means.

As shown in FIG. **1**, construction of the heart valve of the present invention begins with the cutting of a rectangular piece of tissue **10** having a proximal end **12** and a distal end **14** and end edges **15**. The rectangular piece of tissue is preferably cut from a piece of the patient's pericardium obtained at the beginning of the open heart surgery, although other autologous, homologous, or heterologous tissue can be used. The pericardium is advantageously harvested by use of a roughly-sized template placed over the patient's pericardium to guide the surgeon in removing the tissue. Such a template is disclosed in U.S. Pat. No. 5,163,955 assigned to Autogenics, assignee of the present application. This patent is incorporated herein by reference.

The distal end **14** of the tissue piece **10** shown in FIG. **1** is advantageously cut with a scalloped edge **16**, or it can be left straight-edged, as in the alternate embodiment of the invention depicted in FIGS. **9–12**. Such a scalloped edge is preferred because it provides a larger amount of tissue along the edges of the valve leaflets where they coapt. The tissue piece **10** is preferably precisely cut into the proper shape by use of a size-specific cutting die. The size of the required tissue rectangle, and thus of the cutting die, is a function of the diameter of the valve to be replaced and the size of its adjacent inflow and outflow areas. In general, the length of the rectangle required for a valve having inner radius r is approximately $2\pi r$. The radius of the patient's valve is preferably determined by the use of a series of obturators inserted into the annulus by the surgeon. The obturators and their application in sizing the replacement valve are preferably similar to those disclosed in U.S. Pat. No. 5,163,955, assigned to Autogenics and application Ser. No. 08/169,618, filed Dec. 17, 1993, both of which are incorporated herein by reference. The width of the rectangle, i.e. the distance between the distal and proximal ends, is determined by the requirement that the valve leaflets be sufficiently long to coapt at the center of the tissue when it is formed into a cylinder. This consideration will be discussed below.

Dies suitable for cutting the tissue piece **10** are disclosed in the U.S. Pat. No. 5,163,955 and Application Ser. No. 08/169,620, filed Dec. 17, 1993, also assigned to Autogenics and which is incorporated herein by reference. The die (not shown) preferably has a plurality of knives mounted in a solid block of substrate, such as polycarbonate material, at locations corresponding to the desired edges of the tissue piece **10**. The die is preferably provided as an element of a size-specific kit corresponding to the measured size of the valve annulus. The inclusion of the cutting die in a kit advantageously places it within easy reach of the surgeon during the assembly process. The cutting die advantageously includes an opposing surface against which the tissue is held during the cutting process. While the use of a cutting die to cut the tissue piece **10** to the desired shape is preferred, other means may also be used. These include providing a template having an outline of the areas to be cut and cutting the edges with a scalpel, using modified forceps, or laser-based or water jet systems.

The tissue **10** is preferably autologous tissue to prevent an adverse reaction from the patient's immune system. The preferred tissue is pericardium, since this tissue has been found to be satisfactory in practice, but other types of tissue, such as fascia lata or other autologous, homologous, or heterologous tissue, may also prove satisfactory. The tissue is preferably treated by brief immersion in a glutaraldehyde solution. Brief immersion accomplishes the two-fold purpose of making the tissue stiff enough to be used for valve construction in the operating room while preventing it from thickening, shrinking, and calcifying after it has been implanted as a heart valve prosthesis. The preferred glutaraldehyde concentration of the solution is approximately 0.6%, buffered to pH 7.4, since this strength has been found to render the tissue stiff enough for use in valve construction. The immersion time is preferably only a few minutes, typically 5 to 10 minutes, since long-duration immersion may promote calcification of tissue in the resulting valve. Other chemicals, such as glycerol, formaldehyde, or polyglycidyl ether, could also be used as fixing agents.

After being cut into the proper shape and briefly immersed, the tissue **10** is folded at a location **18** distal to its proximal end, as shown in FIG. **2**. The fold forms an inner wall **20** and an outer wall **22**, as is seen in FIG. **3**. As described below, this inner wall **20** forms the valve leaflets. Consequently, the fold location **18** is chosen so that the inner wall **20** has a height sufficient for the valve cusps to completely close.

After folding the tissue **10**, the valve maker applies sets of staples **24**, **25**, **26**, and **27** to the inner and outer walls. The staples **24-27** extend distally from the folded location **18** and are parallel to the end edges of the tissue. These staples join the inner and outer surfaces **20** and **22** together along their height and segment the proximal portion of the tissue **10** into three segments **28**, **30**, and **32**. The valve maker preferably adds three more sets of staples **34**, **36**, and **38** in a curvilinear pattern at the proximal end of the tissue. The curvilinear sets of staples should be tangent to the midpoint of each of the segments **28**, **30**, and **32** and their ends should not extend more than several millimeters above the fold location **16**. The exact amount of extension required for the ends above the fold location **18** depends upon the size of the completed valve.

The sets **34**, **36**, and **38** of staples are advantageously added in the present invention to eliminate areas of stagnation in the blood flow through the valve and thus prevent the formation of blood clots in the valve. The curvilinear sets of staples achieve this objective by sealing off each of the segment corner areas **40**, which would otherwise be the last areas to be purged of blood during the opening and closing of the valve.

The staple sets **24–27**, **34**, **36**, and **38** are preferably applied by well known surgical staplers (not shown) which are provided in the size-specific kit containing the tissue cutting template and die, the size of the staplers varying with the size of the patient's annulus. Stapling the tissue has been found to provide a quick, accurate method of securing the tissue together permanently. The staplers are preferably configured to match the tissue thickness. Other means for securing the inner and outer walls together, such as suturing, could also be employed instead of stapling.

The inner walls **20** of the segments **28, 30,** and **32,** which are fixed at their lower edges to the outer wall **22** of the valve by the staple segments **34, 36,** and **38,** form the cusps of the assembled valve. The provision of the scallops **16** on the top of the inner wall **20** advantageously ensures the availability of a greater amount of tissue along the free edges of the leaflets for better coaption between the commissures of the valve.

After adding the two sets of staple rows **24, 25, 26, 27, 34, 36,** and **36** to the tissue rectangle **10,** the valve maker joins each of the end tissue edges **15a** and **15b** which are parallel to the staple rows **25** and **27.** Other techniques, such as suturing, could, of course, also be used to secure the end edges of the tissue **10** together.

The resulting valve **50,** illustrated in FIGS. **5** and **8,** contains three leaflets **52, 54,** and **56,** which are the inner walls of the segments **28, 30,** and **32.** An important feature of the present invention is the provision of the portion of the outer wall **22** distal to the top of the inner wall **20** as an outflow tract **60,** which can be tailored to the patient's vascular anatomy. This allows the surgeon to cut portions of this outflow tract **60** to precisely tailor it to the anatomy of the patient before implanting the valve in the patient.

As can be seen in FIG. **8,** the valve leaflets **52, 54,** and **56** coapt at edges **62, 64,** and **66.** The outer wall of the valve of the present invention is advantageously tucked opposite the top or distal end of the inner wall at a plurality of locations **68, 70,** and **72** midway along each of the arcs defined by the edges of the leaflets **52, 54,** and **56** and formed by the valve annulus **6.** This important feature reduces the circumference of the outer wall, thus leaving a greater length of tissue on the inner wall than on the outer wall. This achieves the object of preventing the leaflets from adhering to the outer wall of the valve by surface tension and consequently preventing the valve's closure. By pinching the wall at these locations, the top portion of each of the leaflets is displaced from the outer wall, thus preventing adhesion. The outer wall is preferably tucked or pinched by the surgeon and then a suture or, most preferably, a staple, is emplaced to make the pinch a permanent feature of the valve at the locations shown at **68, 70,** and **72.**

In another embodiment of the present invention, illustrated in FIG. **7,** the leaflets are prevented from adhering to the outer wall of the valve by the use of a girdle **74** placed around the midsection of the valve. The girdle is preferably fashioned from synthetic material, cloth, or is cloth-covered. The girdle is placed and secured around the midsection of the valve **50,** constricting it to form valve sinuses **76.** The sinuses **76** provide sufficient curvature to the outer wall of the valve to prevent the leaflets **52, 54,** and **56** from adhering to the wall.

Following the emplacement of either the pinching staples or the girdle in the valve, the surgeon implants the completed valve into the patient, preferably by suturing the proximal end of the valve along a line within 1 mm of the fold **18** into the heart of the patient at location **78,** as shown in FIG. **6.** The surgeon then cuts the distal end of the valve **50** to conform to the patient's vascular anatomy and finally sutures the distal end of the valve into the patient's vein or artery, as, for example, the patient's pulmonary artery at location **80.**

FIGS. **9–12** illustrate a second embodiment of the present invention, in which corresponding numbers denote like parts. The surgeon or technician begins the fabrication of the valve of the second embodiment by cutting a tissue rectangle **10** having a straight lower edge **90.** The subsequent steps in the fabrication and implantation of the valve are identical to those employed in the first embodiment and described in detail above.

The valve of the present invention is very well suited for the low pressure-differential environment characteristic of the pulmonary valve of the patient; however, it could be equally well used in the aortic position or any other position in which an unstented three-leaflet valve would be satisfactory.

It can thus be seen that the valve of the present invention is easily manufactured by a vascular surgeon or technician in an operating-room environment. The size-specific kits containing staplers and cutting dies of the invention also allow the valve of the present invention to be precisely fabricated in a short amount of time.

While embodiments and applications of this invention have been shown and described, it should be apparent that the present disclosure of the preferred embodiment may be changed by a person skilled in the art without departing from the scope of the appended claims.

What is claimed is:

1. A stentless prosthetic heart valve formed from tissue removed from a patient during open heart surgery, said heart valve comprising:

a single piece of autologous tissue substantially entirely forming said heart valve, inner and outer layers of said tissue having a common fold, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together to form an annular, self-supporting heart valve in which the three segments of inner tissue form three coapting valve cusps.

2. A stentless prosthetic heart valve formed from tissue removed from a patient during open heart surgery, said heart valve comprising:

a single piece of autologous tissue substantially entirely forming said heart valve, inner and outer layers of said tissue having a common fold, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, each segment having opposing corners at said fold, said inner and outer layers secured together along an arc formed in each segment, said arc sealing the corners of said segments of tissue from bloodflow through said valve to prevent stagnation of blood in said valve during the operation thereof, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together to form an annular, self-supporting heart valve in which the three segments of inner tissue form three coapting valve cusps.

3. A stentless prosthetic heart valve formed from tissue removed from a patient during open heart surgery, said heart valve comprising:

a single piece of autologous tissue substantially entirely forming said heart valve, inner and outer layers of said tissue having a common fold, the upper end of said inner layer having a straight edge, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together to form an annular, self-supporting heart valve in which the three segments of inner tissue form three coapting valve cusps.

4. A stentless prosthetic heart valve formed from tissue removed from a patient during open heart surgery, said heart valve comprising:

a single piece of autologous tissue substantially entirely forming said heart valve, inner and outer layers of said tissue having a common fold, a distal end of said tissue being cut into a scalloped shape, said inner layer including an upper edge having said distal end scalloped shape and an outer layer of tissue, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together to form an annular, self-supporting heart valve in which the three segments of inner tissue form three coapting valve cusps, whereby said scalloped shape of said upper edge of the inner layer provides greater coaptive surface area for said valve cusps.

5. A stentless prosthetic heart valve formed from tissue removed from a patient during open heart surgery, said heart valve comprising:

a single piece of autologous tissue substantially entirely forming said heart valve, inner and outer layers of said tissue having a common fold, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together to form an annular, self-supporting heart valve in which the three segments of inner tissue form three coapting valve cusps, said outer layer of tissue pinched at three locations opposite the distal end of said inner layer of tissue at a midpoint of each segment of tissue to prevent said valve cusps from adhering to said outer layer of tissue during operation of said valve.

6. The prosthetic heart valve of claim 5 wherein said outer layer of tissue is pinched by emplacement of a single staple at the midpoint of each segment of tissue opposite the distal end of said inner layer of tissue.

7. A stentless prosthetic heart valve formed from tissue removed from a patient during open heart surgery, said heart valve comprising:

a single piece of autologous tissue substantially entirely forming said heart valve, inner and outer layers of said tissue having a common fold, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together to form an annular, self-supporting heart valve in which the three segments of inner tissue form three coapting valve cusps, said prosthetic heart valve including a prosthetic cloth or cloth-covered girdle placed around and constricting the midsection of said valve, said girdle preventing said cusps from adhering to said outer layer of tissue during operation of said valve.

8. A stentless prosthetic heart valve formed from tissue removed from a patient during open heart surgery, said heart valve comprising:

a single piece of autologous tissue substantially entirely forming said heart valve, inner and outer layers of said tissue having a common fold, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, said outer layer of tissue extending distally from the distal end of said inner layer to form an outflow tract, said outflow tract selectively cut to match said patient's vascular anatomy, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together form an annular, self-supporting heart valve in which the three segments of inner tissue form three coapting valve cusps.

9. A stentless prosthetic heart valve formed from removed from a patient during open heart surgery, said heart valve comprising:

a single piece of autologous tissue substantially entirely forming said heart valve, said tissue briefly immersed in a solution containing at least 0.6% glutaraldehyde, inner and outer layers of said tissue having a common fold, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together to form an annular, self-supporting heart valve in which the three segments of inner tissue form three coapting valve cusps.

10. A cardiac valve, comprising:

a self-supporting annular body formed substantially entirely from a single piece of autologous pericardium tissue, said tissue having distal and proximal ends, said distal end of said tissue cut into a plurality of scalloped portions, said tissue folded at a fold to form inner and outer layers of tissue, said inner and outer layers of tissue stapled together at a first set of staples along a plurality of lines extending distally from said fold, said inner and outer layers stapled at a second set of staples in a plurality of arcs at said fold, said inner layer of tissue forming a plurality of valve cusps coapting with one another, said second set of staples preventing areas of bloodflow stagnation from forming during operation of said valve, and said scalloped portions providing improved coaption between said valve leaflets.

11. A cardiac valve, comprising:

a single piece of autologous tissue substantially entirely forming said valve, a self-supporting annular body of said tissue having distal and proximal ends, said tissue folded at a fold location to form a plurality of layers of tissue, said layers of tissue secured together along a plurality of lines extending distally from said fold location of said valve.

12. The cardiac valve of claim 11, wherein said layers are secured together by staples.

13. The cardiac valve of claim 11, wherein said layers are secured together by sutures.

14. The cardiac valve of claim 11, wherein said layers are secured together along a plurality of arcs at said fold location of said valve.

15. The cardiac valve of claim 11, wherein said proximal end of said tissue is cut into a plurality of scalloped portions.

16. The cardiac valve of claim 11, wherein a layer of said tissue is tucked at a plurality of locations around said annular body of tissue to prevent said layers from adhering to each other during operation of said valve.

17. The cardiac valve of claim 16, wherein a layer of said tissue is tucked midway between each of said lines securing said tissue opposite the distal end of the innermost of said layers.

18. The cardiac valve of claim 16, wherein a layer of said tissue is tucked by emplacement of a suture in said outer layer opposite the distal end of the innermost of said layers.

19. The cardiac valve of claim 16, wherein a layer of said tissue is tucked by emplacement of a staple in said outer layer opposite the distal end of the innermost of said layers.

20. The cardiac valve of claim 11, wherein said proximal end of said tissue has a straight edge.

21. The cardiac valve of claim 11, further comprising:

a girdle placed around the outside of the middle section of said valve, said girdle constricting the middle section of said valve to form a sinus, said girdle thereby preventing said layers of said tissue from adhering during operation of said valve.

22. The cardiac valve of claim 11 wherein said tissue is immersed in a solution containing glutaraldehyde.

23. The cardiac valve of claim 11 wherein said tissue is autologous pericardium.

24. The cardiac valve of claim 11 wherein said tissue is autologous fascia lata.

25. A cardiac valve, comprising:

a self-supporting annular body consisting of substantially a single piece of autologous tissue, said tissue having a distal end and a proximal end, said distal end cut along a plurality of scalloped sections, said tissue folded at a location distally from said proximal end to form inner and outer layers, said inner and outer layers of tissue secured along a plurality of lines extending distally from said folded location of said valve to the top of said inner layer, said inner and outer layers of tissue secured at the fold forming said inner and outer layers along a plurality of arcs, each of said arcs tangent at its midpoint to said fold, said outer layer pinched at a plurality of locations opposite the distal end of said inner layer to prevent said inner and said outer layers from adhering to each other during the operation of said valve.

26. A stentless prosthetic heart valve formed from tissue removed from a patient during open heart surgery, said valve comprising a single piece of autologous tissue substantially entirely forming said valve, said valve having an annular configuration in which said tissue has been folded into two layers and said layers have been secured to one another into three outer and inner segments, the inner segments of said annular configuration comprising three coapting valve cusps.

27. A stentless prosthetic heart valve, comprising:

a self-supporting annular body consisting substantially of a single piece of autologous pericardial tissue, said tissue having inner and outer layers, said tissue having a common fold, said layers secured to each other along two lines extending distally from said fold to form three segments of tissue, said folded tissue having opposing ends, said opposing ends being parallel to said two distally extending lines, said opposing ends being joined together to form the annular body of the heart valve in which the three segments of inner tissue form three coapting valve cusps.

28. A stentless prosthetic heart valve, comprising:

a self-supporting annular body consisting substantially of a single piece of autologous pericardial tissue, said tissue having inner and outer layers, said tissue having a common fold, said inner and outer layers of tissue stapled together along a plurality of lines extending distally from said fold to form three segments of tissue, said folded tissue having opposing ends, said opposing ends being parallel to said plurality of distally extending lines, said opposing ends being joined together to form the annular body of the heart valve in which the three segments of inner tissue form three coapting valve cusps.

29. The heart valve of claim 26 wherein said tissue is autologous pericardium.

30. The heart valve of claim 26, wherein said tissue is autologous fascia lata.

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